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(71) Applicant: COGNIS, INC. [US/US]; 2330 Circadian Way,
Santa Rosa, CA 95407 (US).

(72) Inventors: CHRISTIANSON, Teresa ; 208 Eucalyptus
Avenue, Cotati, CA 94931 (US). GODDETTE, Dean ;
806 Carlita Circle, Rohnert Park, CA 94928 (US). LAD-
IN, Beth, Frances ; 4836 Fernglen Drive, Santa Rosa,
CA 95405 (US). LAU, Maria, R. ; 3177 Serra Court,
Fairfield, CA 94533 (US). PAECH, Christian ; 2803 Au-
dubon Court, Santa Rosa, CA 95403 (US). REYNOLDS,
Robert, B. ; 412 Corlano Avenue, Santa Rosa, CA 95404
(US). WILSON, Charles, R. ; 2323 Pacheco Place, Santa
Rosa, CA 95401 (US). YANG, Shiow-Shong ; 1108 Na-
varro Street, Santa Rosa, CA 95401 (US).

(74) Agent: DRACH, John, E.; Henkel Corporation, 140 Ger-
mantown Pike, Suite 150, Plymouth Meeting, PA 19002
(US).

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(57) Abstract

Mutant *B. lentus* DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the *B. lentus* DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

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MUTANT PROTEOLYTIC ENZYMES FROM BACILLUS

BACKGROUND OF THE INVENTION1. Field of the Invention

This invention relates to mutant proteolytic enzymes having improved properties relative to the wild-type enzyme, to genetic constructs which code for the mutant proteolytic enzymes, to methods of predicting mutations which enhance the stability of the enzyme, and to methods of producing the mutant proteolytic enzymes.

2. Description of the Related Art

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various *Bacillus* species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by *Bacillus lentus* for use in detergent formulations having increased protease and oxidative stability over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10 to 60°C in aqueous solutions has been disclosed in copending patent application serial number 07/398,854, filed on

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8/25/89. This *B. lentus* alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a *Bacillus licheniformis* ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the *B. licheniformis* ATCC 53926 alkaline protease gene promoter.

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though *Bacillus subtilisin* proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf stability and oxidative stability, with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including *subtilisin* proteases such as BLAP.

The stability of a protein is a function of its three dimensional structure. A protein folds into a three dimensional conformation based upon the primary amino acid sequence, and upon its surrounding environment. The function and stability of a protein are a direct result of its three dimensional structure.

A large body of information has been published which describes changes in enzyme properties as a result of alterations in the primary amino acid sequence of the enzyme. These alterations can result from random or site specific alterations of the gene which expresses the enzyme using genetic engineering techniques. Random approaches mutagenize total cellular DNA, followed by selection for the synthesis of an enzyme with improved properties. This approach requires neither knowledge of the three dimensional structure of the enzyme, nor any predictive capability on the part of the researcher. Site directed

mutagenesis, on the other hand, requires a rational approach for the introduction of amino acid changes. In this approach one or more amino acids may be replaced by other residues by altering the DNA sequence which encodes the protein. This can be accomplished using

oligonucleotide directed in vitro mutagenesis. The following references teach site-directed mutagenesis procedures used to generate specific amino acid substitution(s): Hines, J.C., and Ray, D.S. (1980) *Gene* 11:207-218; Zoller, M.J., and Smith, M. (1982) *Nucleic Acids Res.* 10:6487-6500; Norrander, J., et al. (1983) *Gene* 26:101-106; Morinaga, Y., et al. (1984) *Bio/Technology* 2:636-639; Kramer, W., et al. (1984) *Nucleic Acids Res.* 12:9441-9456; Carter, P., et al. (1985) *Nucleic Acids Res.* 13:4431-4443; Kunkel, T.A. (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Bryan, P., et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:3743-3745.

A rational approach may or may not require knowledge of a protein's structure. For example, patent application WO 89/06279 describes the comparison of the primary amino acid sequence of different subtilisins while contrasting differences in physical and chemical properties. The primary amino acid sequences of the different subtilisins are aligned for the greatest homology, while taking into account amino acid insertions, deletions, and total number of amino acids.

Currently, the amino acid sequences of at least 10 subtilisin proteases have been published. Eight of these subtilisins were isolated from species of *Bacilli*, and include subtilisin 168 (Stahl, M.L., and Ferrari, E. (1984) *J. Bacteriol.* 158:411-418), subtilisin BPN' (Vasantha, N., et al., (1984) *J. Bacteriol.* 159:811-819), subtilisin Carlsberg (Jacobs, M., et al. (1985) *Nucleic Acids Res.* 13:8913-8926), subtilisin DY (Nedkov, P., et al. (1985) *Biol. Chem. Hoppe-Seyler* 366:421-430), subtilisin amylosacchariticus (Kurihara, M., et al. (1972) *J. Biol. Chem.* 247:5619-5631), subtilisin mesenticopeptidase

(Svendsen, I., et al. (1986) FEBS Lett. 196:228-232), subtilisin 147 and subtilisin 309 (Hastrup et al. (1989) WO 89/06279), subtilisin PB92 (Van Eekelen et al. (1989) EP 0328229), and subtilisin BLAP (Ladin, B., et al. (1990) Society for Industrial Microbiology Annual Meeting, Abstract P60). The remaining two subtilisin sequences are thermitase from the fungus *Thermoactinomyces vulgaris* (Meloun, B., et al. (1985) FEBS Lett. 183:195-200), and proteinase K from the fungus *Tritirachium album limber* (Jany, K.-D., and Mayer, B. (1985) Biol. Chem. Hoppe-Seyler 366:485-492).

Methods for obtaining optimum alignment of homologous proteins are described in Atlas of Protein Sequence and Structure, Vol. 5, Supplement 2 (1976) (Dayhoff, M.O., ed., Natl. Biomed. Res. Found., Silver Springs, MD). This comparison is then used to identify specific amino acid alterations which might produce desirable improvements in the target enzyme. Wells, J.A., et al. (1987) Proc. Natl. Acad. Sci. USA 84:1219-1223, used primary sequence alignment to predict site directed mutations which affect the substrate specificity of a subtilisin. Using the alignment approach WO 89/06279 teaches the construction of mutant subtilisins having improved properties including an increased resistance to oxidation, increased proteolytic activity, and improved washing performance for laundry detergent applications. Patent applications WO 89/09819, and WO 89/09830 teach improvement in the thermal stability of subtilisin BPN' by the introduction of one or more amino acid changes based on the alignment of the primary amino acid sequences of subtilisin BPN' with the more thermal stable subtilisin Carlsberg. From hereon, amino acids will be referred to by the one or three letter code as defined in Table 1.

TABLE 1

One and Three Letter Code for Amino Acids

	A = Ala = Alanine
	C = Cys = Cysteine
5	D = Asp = Aspartic acid or aspartate
	E = Glu = Glutamic acid or glutamate
	F = Phe = Phenylalanine
	G = Gly = Glycine
	H = His = Histidine
10	I = Ile = Isoleucine
	K = Lys = Lysine
	L = Leu = Leucine
	M = Met = Methionine
	N = Asn = Asparagine
15	P = Pro = Proline
	Q = Gln = Glutamine
	R = Arg = Arginine
	S = Ser = Serine
	T = Thr = Threonine
20	V = Val = Valine
	W = Trp = Tryptophan
	Y = Tyr = Tyrosine

Rational mutational approaches may also predict mutations which improve an enzyme property based upon the three dimensional structure of an enzyme, in addition to the alignment of primary amino acid sequences described above. One method for determining the three dimensional structure of a protein involves the growing of crystals of the protein, followed by X-ray crystallographic analysis. This technique has been successfully used to determine several high resolution subtilisin structures such as thermitase (Teplyakov, A.V., et al. (1990) 214:261-279), subtilisin BPN' (Bott, R., et al. (1988) J. Biol. Chem. 263:7895-7906) and subtilisin Carlsberg (Bode, W., et al. (1986) EMBO J. 5:813-818), for example.

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property

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different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) oxidative stability, substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into *Bacillus amyloliquefaciens* subtilisin BPN' after alignment of the primary sequences of BPN' and proteases from *B. subtilis*, *B. licheniformis*, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the *B. amyloliquefaciens* carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 Å X-ray crystal structure of the *B. amyloliquefaciens* protease. Amino acids in the carbonyl hydrolase of *B. amyloliquefaciens* which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for oxidative stability; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover, and lower K_m ; Asp36, Ile107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

WO 88/08028 teaches a method for redesigning proteins to increase stability by altering amino acid residues that

are in close proximity to the protein's metal ion binding site. This application describes the alteration of a calcium ion binding site present within subtilisin BPN' through the substitution, insertion, or deletion of amino acid residue(s) in close proximity to that site so that the electrostatic attraction between the amino acids and the calcium ion is increased. The characterization of the calcium ion binding site is accomplished through the analysis of a 1.3 Å three dimensional structure of subtilisin BPN' using a high resolution computer graphics system. This approach allows the selection of amino acids acceptable for replacing the native amino acids in the protease by first simulating the change using the computer model. This allows for the identification of any problems including steric hindrance prior to the actual construction and testing of the mutant proteases.

US patents 4908773 and 4853871 teach a computer based method for evaluating the three dimensional structure of a protein to select amino acid residues where the introduction of a novel disulfide bond will potentially stabilize the protein. Potentially acceptable amino acid residues can then be ranked, and replaced using computer simulation, prior to the actual construction of the mutant protein using site directed mutagenesis protocols.

Several patent applications combine published data on biochemical stability with computer analysis of three dimensional protease structures in order to predict mutations which stabilize the enzyme. US 4,914,031 and WO 88/08033 and WO 87/04461 teach a method for improving the pH and thermal stability of subtilisin aprA by replacing asparagine residues present in asparagine/glycine pairs. Asparagine/glycine pairs in proteins have been shown to undergo cyclization to form cyclic imide anhydroaspartylglycine (Bornstein, P., and Balian, G. (1977) Methods Enzymol. 47:132-145). This cyclic imide is susceptible to base hydrolyzed cleavage leading to inactivation of the enzyme. Computer analysis of the three

dimensional structure of the aprA protease also predicted that formation of the cyclic imide could lead to protease inactivation resulting from a shift of the side chain of the active site serine. The decision to replace the asparagine residue and not the glycine residue was based upon alignment of the aprA sequence with other subtilisin-like enzymes, cucumisin and proteinase K.

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C.E., and Etson, D. (1969) J. Biol. Chem. 244:5333-5338). EP 0130756, EP 0247647, and US 4,760,025 teach a saturation mutation method where one or multiple mutations are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach mutant proteases exhibiting improved oxidative stability, altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved oxidative stability is achieved by substitution of methionine, cysteine, tryptophan, and lysine residues. These publications also teach that mutations within the active site region of the protease are also most likely to influence activity. Random or selected mutations can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor US 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

WO 8705050 teaches a random mutagenesis approach for construction of subtilisin mutants exhibiting enhanced thermal stability. One or more random mutations are introduced into single stranded target DNA using the chemical mutagens sodium bisulfite, nitrous acid, and formic acid. Subsequently, the mutated DNA is transformed into a Bacillus host and at least 50,000 colonies are screened by a filter assay to identify proteases with improved properties. Site directed mutagenesis can then be

used to introduce all possible mutations into a site identified through the random mutagenesis screen. No method for pre selection of amino acids to be altered is taught.

5 EP 0328229 teaches the isolation and characterization of PB92 subtilisin mutants with improved properties for laundry detergent applications based upon wash test results. It teaches that biochemical properties are not reliable parameters for predicting enzyme performance in
10 the wash. Methods for selection of mutations involve the substitution of amino acids by other amino acids in the same category (polar, nonpolar, aromatic, charged, aliphatic, and neutral), the substitution of polar amino acids asparagine and glutamine by charged amino acids, and
15 increasing the anionic character of the protease at sites not involved with the active site. No method for identifying which specific amino acids should be altered is taught, and no rational mutational approach is taught which is based on alignment of X-ray structures of homologous
20 proteases with different properties.

EP 0260105 teaches the construction of subtilisin BPN' mutants with altered transesterification rate/hydrolysis rate ratios and nucleophile specificities by changing specific amino acid residues within 15 Å of the catalytic
25 triad. Russell, A.J., and Fersht, A.R. (1987) Nature 328:496-500, and Russell, A.J., et al. (1987) J. Mol. Biol. 193:803-813, teach the isolation of a subtilisin BPN' mutant (D099S) that had a change in the surface charge 14-15 Å from the active site. This substitution causes an
30 effect on the pH dependence of the subtilisin's catalytic reaction.

There are a number of different strategies for increasing protein stability. Many of these methods suggest types of substitutions to improve the stability of a
35 protein but do not teach a method for identifying amino acid residues within a protein which should be substituted. From entropic arguments, many types of substitutions have

been suggested such as Gly to Ala and any amino acid to Pro (Matthews, B.W., et al. (1987) Proc. Natl. Acad. Sci. 84:6663-6667). Likewise, while it is clear that increasing the apolar size of an amino acid in the core will add to stability, adverse packing effects may more than compensate for the hydrophobic effect, resulting in a decrease in protein stability (Sandberg, W.S., and Terwilliger, T.C. (1989) Science 245:54-57). Menéndez-Arias, L., and Argos, P. (1990) J. Mol. Biol. 206:397-406, performed a statistical evaluation of amino acid substitutions of thermophilic and mesophilic molecules and proposed that decreased flexibility and increased hydrophobicity in the α -helical regions contributes most towards increasing protein stability. From their data, they formulated a set of empirical rules to improve stability.

Increasing the hydrophobicity of certain side chains has long been suggested as a means to improve protein stability. The hydrophobic exclusion of nonpolar amino acids is the largest force driving protein folding. This has been studied by examining the partitioning of amino acids or amino acid analogs from water to a hydrophobic medium. While the numbers vary depending on the work, these studies generally agree that burying a hydrophobic side chain increases protein stability. For example, Kellis, J.T., Jr., et al. (1988) Nature 333:784-786, estimated that the removal of a methyl group destabilizes the enzyme by 1.1 kcal/mole assuming no other structural perturbations occur. Conversely, this predicts that the addition of a methylene group should add 1.1 kcal/mol if no unfavorable contacts occur. Similarly, Sandberg, W.S., and Terwilliger, T.C. (1989) Science 245:54-57, showed that the effect of removing or adding methylene groups is the sum of the hydrophobic effect and structural distortions. Simply adding buried hydrophobic groups may not increase protein stability because the total effect of adding or deleting a methyl group on the local packing structure must be considered. As the protein interior has a para-crystalline

structure (Chothia, C. (1975) Nature 254:304-308), small distortions in the remainder of the structure resulting from the addition methyl group may exact a high cost and reduce rather than increase stability.

5 Along the same lines, the core of λ repressor has been shown to be amazingly tolerant to apolar amino acid substitutions in a functional assay (Bowie, J.U., et al. (1990) Science 247:1306-1310). It is not clear that this is true for larger proteins. The constraints on the
10 hydrophobic core of a small protein may be less stringent than a larger protein simply due to the volume of the core relative to the number of amino acids which need to pack into the region. As the volume of the hydrophobic core increases, the number of amino acids which must pack
15 together correctly increases, requiring more specific nonlocal interactions.

 It has been recognized that increasing the interior hydrophobicity of a protein as a means of increasing the stability is hampered by the difficulty of determining
20 which positions in the protein will lead to stabilization when substituted (Sandberg, W.S., and Terwilliger, T.C. (1991) Trends Biotechnol. 9:59-63). The methods discussed above provide a means of determining what substitutions to make to improve stability but do not identify which sites
25 in the protein are most important. The present invention provides a method of determining which positions in the protein will lead to stabilization when substituted.

SUMMARY OF THE INVENTION

30 Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein are to be understood as modified in all instances by the term "about".

35 The native or wild-type protease from which the mutant proteases according to the invention are derived is a *B. lentus* alkaline protease (BLAP) obtained from *B. lentus* DSM 5483 having 269 amino acid residues, a molecular mass

of 26,823 daltons, and a calculated isoelectric point of 9.7 based on standard pK values. The BLAP gene is obtained by isolating the chromosomal DNA from the *B. lentus* strain DSM 5483, constructing DNA probes having homology to putative DNA sequences encoding regions of the *B. lentus* protease, preparing genomic libraries from the isolated chromosomal DNA, and screening the libraries for the gene of interest by hybridization to the probes.

Mutant *B. lentus* DSM 5483 proteases have been made which are derived by the replacement of at least one amino acid residue of the mature form of the *B. lentus* DSM 5483 alkaline protease. The sites for replacement are selected from the group consisting of Ser3, Val4, Ser36, Asn42, Ala47, Thr56, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114, His118, Ala120, Ser130, Ser139, Thr141, Ser142, Ser157, Ala188, Val193, Val199, Gly205, Ala224, Lys229, Ser236, Asn237, Asn242, His243, Asn255, Thr268. The replacement amino acid residues are listed in Table 2. The numbering of the mutant proteases is based on the *B. lentus* DSM 5483 wild-type protease as given in the SEQ ID NO:52.

Genes which express the mutant *B. lentus* DSM 5483 proteases according to the invention are made by altering one or more codons of the wild-type *B. lentus* DSM 5483 alkaline protease gene which encode for a protease derived by accomplishing at least one of the amino acid substitutions listed in Table 2.

The protease sites listed in Table 2 are sites predicted to affect thermal and surfactant stability relative to the wild-type protease. These sites are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease (henceforth, the target protein) and a homologous protease (henceforth, the reference protein). The three dimensional coordinates of the wild-type protease are probed with an uncharged probe molecule to produce a probe-accessible surface which has an external surface the

interior of which contains one or more probe-accessible internal cavities. The amino acids of the reference protein having side chains lying outside the solvent-accessible surface or inside the internal cavities of the target protein are identified by aligning the three dimensional coordinates of the target protein and the reference protein.

Proteins having greater thermal and surfactant stability are produced by replacing the amino acid in the target protein if the amino acid in the target protein can be changed without creating unacceptable steric effects. The amino acid in the target protein is altered by site directed mutagenesis of the gene which expresses the target protein.

Genetic constructs are made which contain in the direction of transcription a promoter, ribosomal binding site, initiation codon and the major portion of the pre region of the *Bacillus licheniformis* ATCC 53926 alkaline protease gene operably linked to a portion of the pre region and all of the pro and mature regions of the *Bacillus lentus* DSM 5483 alkaline protease gene followed by a 164 bp DNA fragment containing the transcription terminator from the ATCC 53926 alkaline protease gene. The *Bacillus lentus* DSM 5483 alkaline protease gene is altered to produce a mutant gene which encodes for a protease derived by accomplishing at least one of the amino acid substitutions listed in Table 2. Mutant protease is made by fermenting a *Bacillus* strain transformed with a genetic construct containing a mutated *Bacillus lentus* DSM 5483 alkaline protease gene.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the atomic coordinates for *Bacillus lentus* alkaline protease (BLAP) to 1.4 Å resolution.

Figure 2 shows the restriction map for plasmid pCB13C which contains a hybrid gene fusion between the *Bacillus licheniformis* ATCC 53926 protease gene and the *Bacillus lentus* DSM 5483 BLAP gene. The promoter, ribosomal binding

site and presequence (P-53926) from ATCC 53926 were fused to the pro- and mature sequence of the BLAP gene. The transcription terminator of ATCC 53926 (T-53926) was appended to the BLAP coding region.

5 Figure 3 shows the restriction map for plasmid pMc13C which is derived from pMac5-8 and contains the BLAP gene and carries an amber mutation in the Ap^R gene which renders it inactive.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

10 One aspect of the invention relates to mutant proteolytic enzymes which have superior thermal stability and surfactant stability relative to the wild-type protease as determined by laboratory tests. The mutant proteases according to the invention are those derived by the replacement of at least one amino acid residue of the
15 mature *Bacillus lentus* DSM 5483 alkaline protease wherein said one amino acid residue which is selected from the group consisting of Ser3, Val4, Ser36, Asn42, Ala47, Thr56, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114,
20 His118, Ala120, Ser130, Ser139, Thr141, Ser142, Ser157, Ala188, Val193, Val199, Gly205, Ala224, Lys229, Ser236, Asn237, Asn242, His243, Asn255, Thr268 is replaced with the amino acid residues listed in Table 2. Table 2 shows the identity and position of the wild-type amino acid and the
25 amino acid residue(s) which replace it in the mutant protein. For example, the first entry in Table 2 shows Ser3, a serine residue at position 3 which can be replaced by threonine (abbreviated as T using the one letter code for amino acids) or any small amino acid. A small amino
30 acid is defined as glycine, alanine, valine, serine, threonine or cysteine. A small hydrophobic amino acid is defined as glycine, alanine, threonine, valine or isoleucine. A charged amino acid is defined as lysine,

arginine, histidine, glutamate or aspartate. The
 abbreviation a.a. stands for "amino acid" residue.

TABLE 2

	<u>Residue</u>	<u>Replacement Amino Acid</u>
5	Ser3	T or any small, hydrophobic a.a.
	Val4	I, S or any small a.a.
	Ser36	A, T or any small a.a.
	Ser42	F, A, T, V, I, Y
	Ala47	W or any small a.a. except A
10	Thr56	V, S or any small, hydrophobic a.a.
	Thr69	R, A or any charged a.a.
	Glu87	R, M or any charged a.a.
	Ala96	I, N, S or any small, hydrophobic a.a.
	Ala101	T, S or any small, hydrophobic a.a.
15	Ile102	W or any small a.a. except P
	Ser104	T or any small, hydrophobic a.a.
	Asn114	S, Q or any small, hydrophobic a.a.
	His118	F or any a.a. except P and W
	Ala120	V or any small, hydrophobic a.a.
20	Ser130	A, T or any small, hydrophobic a.a.
	Ser139	A, T, Y or any a.a. except P and W
	Thr141	W or any a.a. except P
	Ser142	A, T or any small, hydrophobic a.a.
	Ser157	T or any small, hydrophobic a.a.
25	Ala188	P or any small, hydrophobic a.a.
	Val193	M or any small, hydrophobic a.a.
	Val199	I or any small, hydrophobic a.a.
	Gly205	V or any small, hydrophobic a.a.
	Ala224	V or any small, hydrophobic a.a.
30	Lys229	W or any a.a. except P
	Ser236	A, T or any small, hydrophobic a.a.
	Asn237	A, N, Q, M or any small, hydrophobic a.a.
	Asn242	A, N, Q, M or any small, hydrophobic a.a.
	His243	A, N, Q, M or any small, hydrophobic a.a.
35	Asn255	P or any small, hydrophobic a.a.
	Thr268	V or any small, hydrophobic a.a.

The amino acid sequences of the preferred proteolytic enzymes are given in SEQ ID NO:1 to SEQ ID NO:51. The preferred mutated *B. lentus* DSM 5483 proteases which are encoded for by genes according to the invention as disclosed above are given in SEQ ID NO: 53 to 105. These proteases are produced by bacterial strains which have been transformed with plasmids containing a native or hybrid gene, mutated at one or more nucleotide base pairs by known mutagenesis methods. These mutant genes encode for proteases in which selected amino acid residues have been substituted for by other amino acids.

The mutant proteases according to the invention are listed in Table 3.

Table 3

	Mutation	<u>Temperature Stability</u>		<u>SDS Stability</u>	
		50°C, pH 11.0 t _{1/2} (min)	60°C, pH 10.0 t _{1/2} (min)	pH 10.5, 50°C t _{1/2} (min)	pH 8.6, 50°C t _{1/2} (min)
20	S3T, V4I, A188P, V193M, V199I	120	67	3.2	12
	S3T, A188P, V193M, V199I	95	60	3.75	18.5
	V4I, A188P, V193M, V199I	72	39	1.75	3.75
	S139Y, A188P, V193M, V199I	69	33	1.4	4.6
25	S130T, S139Y, A188P, V193M, V199I	64	22	2	6.3
	A188P, V193M, V199I	55	23.5	3.0	12.5
	S3T, A188P, V193M	54	21	1.5	3.4
	S157T	52	17.5	1.2	0.95
	A188P, V193M	50	27	2.5	7.25
30	A188P	48	19	1.4	2.8
	S3T, V4I, A188P, V193M	43	21	1.4	3.7
	V193M	42	16.6	1.2	3.0
	S104T	42	8	1.0	1.8
	T69V	41	12.3	0.8	1.8
35	V4I, A188P, V193M	40	19	1.25	2.7
	A224V	39	15	0.9	1.1
	V199I	38.5	11.6	1.0	2.0
	V4I	32.5	10	0.75	1.0
	S3T	32	6.6	1.2	2.8
40	S139Y	26	8.8	1.0	2.0
	N242A	26	7.4	0.9	1.9
	S236T	25.5	8.4	1.0	2.0
	S36A	23.8	8.6	0.9	1.8

TABLE 4 (cont.)

Mutation	Temperature Stability		SDS Stability	
	50°C,	60°C,	pH 10.5,	pH 8.6,
	pH 11.0	pH 10.0	50°C	50°C
	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)
H243A	23	5.9	0.8	1.7
A101T	23	4.7	0.5	2.75
S236A	23	5.1	0.8	1.3
E87R	22.5	9.0	0.4	1.2
5 N114S	22	7.9	1.1	1.3
A47W	21	7.2	0.9	1.05
A120S	20.5	8.4	0.9	1.4
T56V	20	8.5	0.8	0.7
A120V	20	11.8	0.65	1.9
10 G205V	20	6.8	1.1	2.8
S130A	20	8.8	0.4	1.0
S130T	20	7.2	0.4	1.1
A96I	19	12	1.0	1.4
S104T, S139Y, A224V	18	9.5	1.0	1.8
15 S139A	18.5	7.8	0.5	0.8
S142T	17.5	11.5	0.9	1.7
S139T	16.5	4.3	0.5	0.8
I102W	16.5	7.2	0.7	1.6
A96N	16	6	0.9	0.95
20 N42F	16	5.9	1.0	1.4
S142A	16	9	1.0	1.7
H118F	15.8	5.1	1.0	1.3
N237A	15	7.8	0.67	1.3
N255P	15.0	5.3	1.2	1.25
25 T141W, N237A	14	5.4	0.33	1.1
T268V	14	3.8	0.75	1.1
K229W	13.4	4.6	1.0	1.4
T141W	12	6.5	0.6	1.4
wildtype	12.0	3.0	0.8	1.6

30

Any of the proteases listed in Table 3 will exhibit greater stability in some manner than the wild-type protease BLAP. The entries under the "Mutation" heading of Table 3 shows the identity of the wild-type amino acid (using the one letter code), its position, and the amino acid which

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replaces it in the mutant protease. For example, S3T signifies that the serine at position 3 of the mature protease is replaced with a threonine. Some of the preferred mutant proteases are single replacements at specific locations such as a protease wherein valine at position 4 is replaced by isoleucine to specific combinations of replacements such as a protease wherein threonine at position 141 is replaced by tryptophan and asparagine at position 237 is replaced by alanine. The latter protease containing two replacements is one of only a number of possibilities.

The preferred mutant proteases according to the invention are identified as: (S3T, V4I, A188P, V193M, V199I); E87R; (S3T, A188P, V193M, V199I); N114S; (V4I, A188P, V193M, V199I); A47W; (S139Y, A188P, V193M, V199I); A120S; (S130T, S139Y, A188P, V193M, V199I); T56V; A120V; (A188P, V193M, V199I); G205V; (S3T, A188P, V193M); S130A; S130T; S157T; A96I; (S104T, S139Y, A224V); S139A; S142T; S139T; I102W; V193M; A96N; N42F; S142A; H118F; N237A; N255P; (T141W, N237A); T268V; K229W; T141W; (A188P, V193M); V4I; S3T; S139Y; N242A; S236T; S36A; H243A; A101T; S236A; A188P; (S3T, V4I, A188P, V193M); V193M; S104T; T69V; (V4I, A188P, V193M); A224V; V199I. The system used to designate the above preferred proteases first lists the amino acid residue in the mature form of the *B. lentus* DSM 5483 alkaline protease at the numbered position followed by the replacement amino acid residue using the one letter codes for amino acids. For example, V193M is a protease in which valine has been replaced by methionine at position 193 of the mature *B. lentus* DSM 5483 alkaline protease. A mutant protease identified by more than one such designation is a mutant protease which contains all of the indicated substitutions. For example, (A188P, V193M) is a protease in which valine has been replaced by methionine at position 193 of the mature *B. lentus* DSM 5483 protease and alanine at position 188 has been replaced by proline.

Mutant forms of the *B. lentus* DSM 5483 alkaline protease are prepared by site-specific mutagenesis of DNA encoding the mature form of either wild-type BLAP, or a mutant BLAP. The DNA fragment encoding the mature form of wild type BLAP was prepared using plasmid pCB13C. Plasmid pCB13C contains a hybrid fusion between the *B. licheniformis* ATCC 53926 protease gene and the *B. lentus* DSM 5483 BLAP gene, shown in Figure 2. Specifically, this hybrid fusion contains DNA encoding the promoter, ribosomal binding site, and 21 residues of the pre sequence from the ATCC 53926 protease gene fused to a DNA sequence encoding the last five residues of the BLAP pre sequence and all of the pro and mature residues of BLAP. This fusion is referred to as the *Cla*I fusion because this restriction site is located at the juncture between the ATCC 53926 and DSM 5483 DNA's. A new *Cla*I restriction site had to be introduced into the ATCC 53926 alkaline protease gene near to the junction of the pre and pro sequences. The *Cla*I site was introduced into the ATCC 53926 alkaline protease gene by using a polymerase chain reaction (PCR) to amplify a DNA fragment containing sequence information from the N-terminal part of the ATCC 53926 alkaline protease gene. The amplified fragment included the ATCC 53926 alkaline protease promoter, ribosomal binding site, initiation codon, and most of the pre sequence. This 292 bp DNA fragment was flanked by *Ava*I and *Cla*I restriction sites at its 5' and 3' ends, respectively. The BLAP gene already contained a naturally occurring *Cla*I site at the corresponding position. Analysis of the DNA sequence across the fusion of the ATCC 53926 and BLAP genes confirmed the expected DNA and amino acid sequences.

Before any mutagenesis can be carried out, the gene is subcloned into the mutagenesis vector pMa5-8. This is accomplished by synthesizing a DNA fragment containing the *Cla*I fusion gene and the ATCC 53926 transcription terminator as a *Sal*I cassette using the PCR. The PCR was carried out using conditions as described by the

5 manufacturer (Perkin Elmer Cetus, Norwalk, CT.). In the PCR, two synthetic oligonucleotides bearing *Sal*I sites are used as primers and *Escherichia coli* vector pCB13C DNA as a template. After cutting the PCR product with *Sal*I, this
10 fragment is cloned into the mutagenic plasmid pMc5-8 which has previously been cut with *Sal*I and dephosphorylated with bacterial alkaline phosphatase. Plasmids pMc5-8, and pMa5-8 described below were obtained from H.-J. Fritz and are described by Stanssens, P., et al. (1989) *Nucleic Acids Res.* 17:4441-4454. *Sal*I sites are chosen to allow the PCR
15 fragment to be cloned into pMc5-8 in both orientations. The ligation mix is transformed into *E. coli* WK6. Chloramphenicol resistant (Cm^R) transformants are screened for the presence of an insert and a correct plasmid construct pMc13C is identified as shown in Figure 3. Once the gene is cloned into the pMc vector and desirable sites for mutation are identified, the mutation(s) is introduced using synthetic DNA oligonucleotides according to a modification of a published protocol (Stanssens, P., et al. (1989) *Nucleic Acids Res.* 17:4441-4454). The
20 oligonucleotide containing the mutation(s) to be introduced is annealed to a gapped duplex (gd) structure which carries the BLAP gene on a segment of single stranded (ss) DNA. The gapped duplex can be formed by annealing linear ss DNA from pMc13C with denatured and restricted pMa5-8 DNA.
25 Plasmid pMa5-8 contains an active ampicillin resistance gene but has an inactivating point mutation in the chloramphenicol resistance gene, whereas plasmid pMc13C contains, in addition to an intact BLAP gene, an active chloramphenicol resistance gene, but has an inactivating
30 point mutation in the ampicillin resistance gene. The annealed product is the gd DNA which is a double stranded heteroduplex with a ss DNA gap spanning the entire cloned BLAP gene. The mutant oligonucleotide is able to anneal to
35 homologous ss BLAP DNA within the gap and the remaining gap is filled in by DNA polymerase I (Klenow fragment) and ligated using T4 DNA ligase, purchased from New England

Biolabs Inc., Beverly, Ma. The mutagenic efficiency of such a system can be improved by the use of Exonuclease III (Exo III) purchased from New England Biolabs Inc., Beverly, MA. Exo III is an exodeoxyribonuclease that digests double stranded DNA from the 3' end. As a free 3' end is required, closed circular ss DNA or ds DNA is unaffected by this enzyme. A subsequent treatment of the product of the fill-in reaction with Exo III removes any species with only partially filled gaps. This significantly improves the mutagenic efficiency and is the preferred mutagenesis method. The product of the fill-in reaction is then transformed into a repair deficient *E. coli* strain such as WK6mutS and ampicillin resistant transformants (Ap^R) are selected. Replication of the transformed heteroduplex phasmid results in two different progenies. One progeny contains the wild type BLAP gene and the intact chloramphenicol resistance gene, but an inactive ampicillin resistance gene. The other progeny contains a BLAP gene carrying the mutation of interest and is resistant to ampicillin but not to chloramphenicol.

Selection of Ap^R, Cm^S mutant transformants with ampicillin is not sufficient to stop some background growth of the Ap^S, Cm^R progeny carrying the wild type BLAP gene. Therefore, it is necessary to perform a second transformation into *E. coli* using plasmid DNA prepared from the Ap^R transformants of the WK6mutS strain. This second transformation uses a low plasmid concentration with a large number of recipient cells of a suppressor deficient strain of *E. coli* such as WK6. This approach decreases the likelihood of a recipient cell receiving plasmid DNA from both progeny. Ap^R transformants are selected and plasmid DNA from several transformants is isolated and screened for the presence of the mutation. The pMa mutant derivative of the first mutagenesis round can be used for a second round of mutagenesis by preparing ss DNA of that species and annealing it to XbaI/HindIII restricted and denatured DNA of pMc5-8. Plasmid pMc5-8 is identical to pMa5-8 except

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that it contains an active chloramphenicol resistance gene and an inactive ampicillin resistance gene. The general procedure is the same as that described above.

The mutant BLAP proteases can be produced by transferring the mutant BLAP genes from their particular *E. coli* pMa13C derivative vector into a plasmid vector which can replicate in *Bacillus*. To accomplish this, the mutant BLAP genes are separated from their pMa13C plasmids by digestion with the restriction endonucleases *Ava*I and *Sst*I, followed by ligation to the larger *Ava*I/*Sst*I fragment from either plasmid pH70 or pC51. These *Ava*I/*Sst*I fragments from pH70 and pC51 include the DNA sequences necessary for replication in *Bacillus* and encode either kanamycin resistance (Km^R) or tetracycline resistance (Tc^R), respectively. Plasmid pH70 is constructed by cloning the ATCC 53926 alkaline protease gene carried on a *Eco*RI/*Bam*HI DNA fragment into the Km^R plasmid pUB110 between the *Eco*RI and *Bam*HI sites. Plasmid pC51 is constructed by cloning the ATCC 53926 protease gene carried on a *Eco*RI-*Bam*HI fragment into the Tc^R plasmid pBC16 between the *Eco*RI and *Bam*HI sites. The larger *Ava*I-*Sst*I fragment from either pH70 or pC51 used for cloning the mutant BLAP genes is first purified from other DNA fragments by high pressure liquid chromatography (HPLC) on a Gen-Pak FAX column (Waters, Milford, MA). The column is 4.6 mm by 100 mm in size and contains a polymer-based high performance anion-exchange resin. Conditions for elution of the DNA are a flow rate of 0.75 ml/min with a gradient of Buffer A (25 mM tris(hydroxymethyl)aminomethane (Tris) pH 8.0 containing 1 mM disodium ethylenediamine tetraacetic acid (EDTA)) and Buffer B (25 mM Tris pH 8.0, 1 mM EDTA, 1 M NaCl) starting at 50% each and reaching a final concentration of 30% Buffer A and 70% Buffer B.

After ligation the mutant BLAP plasmids are transformed into *B. subtilis* DB104. The genes encoding the major alkaline and neutral proteases present in this strain have been inactivated (Kawamura, F., and Doi, R.A.

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(1984) J. Bacteriol. 160:442-444). Cells of *B. subtilis* DB104 transformed by these plasmids grow on a nutrient-skim milk agar in the presence of either kanamycin or tetracycline. Transformants of DB104 that manufacture mutant protease are identified by the formation of clear zones of hydrolysis in the skim milk. Confirmation that the protease-producing transformants carry a plasmid-borne BLAP gene with the desired mutation(s) is accomplished by purifying plasmid DNA from a culture of each transformant. The plasmid DNA is purified away from cell protein and chromosomal DNA by SDS-salt precipitation followed by chromatography over a Qiagen ion-exchange column (Qiagen Corporation, Studio City, CA). *Ava*I-*Sst*I digested plasmid DNAs from different transformants are compared with *Ava*I/*Sst*I-digested derivatives of plasmid pH70 or pC51 known to carry an intact BLAP gene. Restriction digests of these plasmids are compared by agarose gel electrophoresis to identify plasmids that have the proper-sized *Ava*I/*Sst*I DNA fragments. Selected plasmid DNAs are then sequenced across the region of the expected BLAP mutation(s) to confirm that the desired mutation(s) are present. One or more clones of each BLAP mutation are stored frozen in 15% glycerol at -70°C and also cultivated in shake flasks (Example 4, Production of Proteases) to produce mutant protease for characterization.

Another aspect of the invention provides a computer based method for identifying the sites which affect the storage, thermal, SDS and pH stability of a protein. This method is based on the hypothesis that protein stability may be enhanced by decreasing the volume of internal cavities and improving surface packing of amino acid side chains. The interior of a protein contains many apolar amino acids which are tightly packed into a nearly crystalline state. One way in which these interior amino acids affect protein stability is through packing effects. These include van der Waal interactions, distortion of the remainder of the protein and electrostatic effects.

Packing effects have been studied by measuring the contribution of methyl groups in the interior of a protein to the overall stability of the protein. It has been estimated that the removal of a methyl group from the interior of a protein destabilizes it by about 1.1 kcal/mol assuming no other perturbations occur (Kellis, J.T., Jr., et al. (1988) Nature 333:784-786). However, the inverse may not be true. Simply adding buried hydrophobic groups may not increase protein stability because the total effect of adding or deleting a methyl group on the local packing structure must be considered. As the protein interior has a para-crystalline structure (Chothia, C. (1975) Nature 254:304-308), small distortions in the remainder of the structure resulting from the addition methyl group may exact a high cost and reduce rather than increase stability.

While it is known in the art to make certain substitutions which may affect protein stability, there is no known way of identifying which sites in the protein will lead to stabilization when substituted. For example, it has been suggested that protein stability would be increased if alanine were substituted for glycine or serine; or if threonine were substituted for serine (Matthews, B.W., et al. (1987) Proc. Natl. Acad. Sci. 84:6663-6667); or if proline were substituted for glycine. However, the sites in which one or more of these substitutions should be made has been so far unpredictable. Other methods depend on comparisons of the amino acid sequences of different but related proteins. However, this does not show which sites are important to stability, only which positions are different.

There are two computer based methods for identifying the sites which affect the stability of a protein according to the invention.

In the first method for identifying sites which affect the stability of protein, the first step comprises generating a probe-accessible surface by analyzing the

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target protein coordinates with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å. It is important that no water molecules be included in the protein structure during this analysis. The second step of this method is the identification of the amino acids which form the boundaries of the internal cavities. These amino acids comprise a set of positions which, if mutated, may increase the stability of the protein. An increase in stability can be achieved by amino acid substitutions which decrease the volume of the internal cavities.

The molecular modeling program QUANTA (trademark of Polygen Corporation, 200 Fifth Ave., Waltham, MA 02254) was used to calculate probe-accessible surfaces as well as perform the alignment of the three dimensional coordinates of the proteins. These functions can be carried out equally well by other molecular modeling programs which are also commercially available. The following is a list of commercially available programs which can also be used to calculate probe-accessible surfaces: Insight or InsightII (trademark of Biosym Technologies, Inc., 10065 Barnes Canyon Road - Suite A, San Diego, CA 92121), BIOGRAF (trademark of Biodesign, Inc., 199 S. Los Robles Ave., #270, Pasadena, CA 91101) or Sybyl (trademark of Tripos Associates, 1699 S. Hanley Road, St. Louis, MO 63144)

The probe-accessible surface referred to in step 1 of the first method can be generated in several ways (Richards, F.M. (1977) Annu. Rev. Biophys. Bioeng. 6:151-176): A spherical probe of radius R (0.9 to 2.0 Å) is allowed to roll on the outside of a molecule while maintaining contact with the van der Waal surface. The surface defined by the center of the probe is defined as the probe-accessible surface. Alternatively, a similar surface can be generated by increasing the van der Waal radii of all the atoms in a protein by the radius of the probe. Overlapping surfaces are eliminated and the remaining surface represents the probe-accessible surface. In the preferred embodiment, a three-dimensional box of

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dimensions 50x50x50 Å with a 1 Å grid size in all three dimensions (x, y, and z) is centered on the center of mass of the target protein coordinates. Most preferably, the dimensions of the probe map are adjusted such that all of the protein atoms fall within the probe map's bounds. The grid size of 1 Å provides a sufficiently high resolution to clearly define the probe-accessible surface although another grid size could be used, ranging from 0.5 to 3.0 Å. An uncharged probe molecule is positioned at each grid point and the energy of interaction between the probe and the target protein atoms is determined. The energy of nonbonded interaction (E_{nb}) contains only the van der Waal component such that

EQUATION (1)

$$E_{nb} = \sum_{\substack{\text{nonbonded} \\ i,j \text{ pairs}}} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r} \right)^{12} - \left(\frac{\sigma_{ij}}{r} \right)^6 \right]$$

where r is the nonbonded distance, ϵ_{ij} is the dispersion well depth and σ_{ij} is the Lennard-Jones diameter. The result is a map consisting of a box with energy values at each grid point. This map can be contoured at a particular energy value to generate surfaces which correspond to the solvent accessible surface and internal cavities (Goodford, P.J. (1985) J. Med. Chem. 28: 49-857). The value at which to contour the maps can vary depending on the particular radius used and the parameters used to define the probe molecule and the particular method used to generate the probe. The preferred embodiment is to use a probe radius of 0.9 Å and contour the surface at 10 kcal/mol.

The external surface of the probe-accessible surface is also known as the solvent-accessible surface. Probe-accessible surfaces inside of the solvent accessible surface are defined as internal cavities and represent cavities large enough to accommodate a molecule with a

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radius equal to the probe radius. The presence of such a cavity on the inside of a protein does not imply that the cavity will in fact be filled by one or more solvent molecules.

5 The second step of the method for identifying sites which affect the stability of a protein is the identification of the amino acids which form the internal cavities. The internal cavities are defined by the amino acids which make up its boundaries. These amino acids
10 comprise a set of positions which, if mutated, may increase the stability of the protein.

 In a second method for identifying sites which affect the stability of a protein, the first step comprises generating a probe-accessible surface by analyzing the
15 target protein coordinates with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å. It is important that no water molecules be included in the protein structure during this analysis. This step is the same as the first step of the method set forth above.

20 The second step involves aligning the three dimensional structure of the target protein and a reference protein by moving the three dimensional coordinates of the reference protein into the coordinate frame of the target protein. The reference protein is usually chosen so that a
25 high degree of similarity exists between it and the target protein so that packing differences between the target and reference protein which potentially affect the stability of the target protein can be identified. The reference protein can be any protein for which a three dimensional structure
30 is available which is homologous to the target protein. Examples of such proteins include but are not limited to subtilisin Carlsberg, subtilisin BPN', proteinase K, and Thermitase. When the target protein is BLAP, one
35 preferred reference protein is Thermitase. Thermitase is an extra-cellular subtilisin-like serine protease isolated from *Thermoactinomyces vulgaris* (Frömmel, C., et al. (1978) Acta Biol. Med. Ger. 37:1193-1204). The protein amino acid

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sequence of thermitase is 42% identical to BLAP. The high degree of similarity between these two proteins provides an ideal system with which to examine packing differences that affect BLAP stability. In this second step the three dimensional structures of Thermitase and BLAP are aligned using the computer program QUANTATM. The three dimensional alignment is carried out by first aligning the primary sequences of the two proteins to determine which amino acids are equivalent. This is accomplished using FASTA (Myers, E.W., and Miller, W. (1988) Comput. Applic. Biosci. 4:11-17; Pearson, W.R., and Lipman, D.J. (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448). Based on this alignment of the primary sequence, residues are matched for subsequent alignment of the three dimensional structures using MULTISO (Sutcliffe, M.J., et al. (1987) Protein Eng. 1:377-384; Kabsch, W. (1976) Acta Cryst. A32:922-923). This program uses one structure as fixed coordinates (the target protein coordinates) and then rotates and translates a second structure (the reference protein coordinates) so as to give the smallest root mean squared (r.m.s.) deviation between the two sets of three dimensional coordinates. For example, the alignment of the BLAP and thermitase three dimensional coordinates results in an r.m.s. deviation between equivalent α -carbons of 0.8 Å. This demonstrates that the amino acid sequences of BLAP and thermitase fold into three dimensional structures which are extremely similar.

In the third step, the alignment of the three dimensional structures is used to identify sites which affect the stability of the target protein. This can be accomplished by a variety of methods. Using a computer program designed to display protein structures and surfaces such as QUANTATM, the structure of the reference protein can be displayed with the probe-accessible surface. The combined display of the reference protein and probe-accessible surface can then be visually examined to determine which amino acids in the reference protein fall

outside of the solvent-accessible surface or inside internal cavities. An alternative method which can be used comprises coloring the atoms of the reference protein by determining whether amino acids in the reference protein fall outside of the solvent-accessible surface or inside internal cavities. The probe-accessible surface map (probe map) was used to color the atoms in the transformed subtilisin BPN' structure. In order to color each atom, an energy value needs to be interpolated from the probe map at each atomic coordinate.

The probe map consists of three dimensional grid with an energy value (E) at each grid point. In the preferred embodiment, the probe map is a 50x50x50 Å box centered on the center of mass of the protein with a 1 Å grid unit in all three dimensions (x, y, and z). In its optimal conception, the size of the probe map is adjusted such that all of the protein atoms fall within the probe map's bounds. The energy value at each protein atom position was approximated by interpolating from the energy values from the surrounded eight grid points in the probe map. Given the energy value at each point from the probe map, the grid spacing, and the atomic coordinate, it is a simple matter for any one skilled in the art to interpolate an energy value at each atomic coordinate.

In one such method, an energy value of zero is assigned arbitrarily if an atom falls outside the bounds of the map. From a given atomic coordinate (x,y,z), the eight closest grid points from the probe map which surround (x,y,z) are identified such that $(x_1 < x < x_2)$, $(y_1 < y < y_2)$, and $(z_1 < z < z_2)$. The eight grid points are then A (x_1, y_1, z_1) , B (x_1, y_1, z_2) , C (x_1, y_2, z_2) , D (x_1, y_2, z_1) , E (x_2, y_1, z_1) , F (x_2, y_1, z_2) , G (x_2, y_2, z_2) , and H (x_2, y_2, z_1) . The energy value (E) at a given grid point such as (x_1, y_1, z_1) is then $E(x_1, y_1, z_1)$ or equivalently E_A . The energy at a specific atomic coordinate $E_{(x,y,z)}$ can be interpolated from the probe map given the eight nearest surrounding grid points (A through H, as

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described above) and the value at each grid point (E_A through E_H). The equation which was used for calculating the energy at specific atomic coordinates, $E_{(x,y,z)}$, is shown in Equation (2). The energy value at each coordinate can then be stored and used to display the molecule.

EQUATION (2)

$$E_{(x,y,z)} = \left(\frac{x-x_1}{x_2-x_1} \right) (E_o - E_k) + E_k$$

where

$$E_o = \left(\frac{y-y_1}{y_2-y_1} \right) (E_i - E_j) + E_j; \text{ and } E_k = \left(\frac{y-y_1}{y_2-y_1} \right) (E_j - E_l) + E_l;$$

and where

$$E_i = \left(\frac{z-z_1}{z_2-z_1} \right) (E_f - E_g) + E_g; \quad E_j = \left(\frac{z-z_1}{z_2-z_1} \right) (E_g - E_h) + E_h;$$

$$E_l = \left(\frac{z-z_1}{z_2-z_1} \right) (E_h - E_a) + E_a; \quad E_m = \left(\frac{z-z_1}{z_2-z_1} \right) (E_c - E_d) + E_d;$$

The protein atoms were colored on the basis of this interpolated energy value. The protein was displayed using QUANTATM and atoms with interpolated energies below 10 kcal/mol were colored as red. Atoms with interpolated energies above 10 kcal/mol were colored green. Visual inspection allowed identification of side chains which penetrated the solvent accessible surface or penetrated internal cavities.

There are also two computer based methods for increasing the stability of a protein. The first method comprises the steps of: (1) generating a probe-accessible surface of said target protein by probing the coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal

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cavities; (2) identifying the amino acids which make up the boundaries of the internal cavities, wherein said amino acids comprise a set of sites which when mutated increase the stability of the protein; (3) identifying an amino acid mutation which would decrease the volume of said internal cavities; (4) determining if said amino acid in said target protein can be changed without creating unacceptable steric interactions; (5) replacing the amino acid in said target protein by site-directed mutagenesis of the gene which expresses said target protein.

The first two steps of the above first method for improving the stability of a protein are the same as those disclosed above for the first computer based method for identifying the sites which affect the stability of a protein.

In step (3) an amino acid identified in step (2) is examined with the goal of identifying a mutation which would decrease the volume of said internal cavity. The size, shape and position of said internal cavity often defines and limits what mutations are acceptable and allowable given the distinct shape and size of each individual amino acid side chain. However, as a particular site in the protein has been identified for mutation, appropriate mutations can be also be determined by applying any of the various heuristics which define generally acceptable mutations (Matthews, B.W., et al. (1987) Proc. Natl. Acad. Sci. 84:6663-6667; Menéndez-Arias, L., and Argos, P. (1990) J. Mol. Biol. 206:397-406; Sandberg, W.S., and Terwilliger, T.C. (1991) Trends Biotechnol. 9:59-63; Bordo, D., and Argos, P. (1991) J. Mol. Biol. 217:721-729).

In step (4) a determination is then made if the amino acid identified for change in the target protein can be mutated or changed without creating a conformation of the target protein having unacceptable steric interactions. The separation distance between two atoms considered unacceptably short is some percentage of the sum of the van der Waal radii of the two atoms in question. Values of 90-

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95% of the sum of the van der Waal radii are common though others could be used. Common atoms between the original and replacement amino acid side chain are located and fixed in the same position. The new amino acid is rotated to find the position with the least number of close contacts or unacceptable steric interactions (distances shorter than physically reasonable). The separation distance at which two atoms are considered unreasonably short is some percentage of the sum of the van der Waal radii of the two atoms in question. Values of 90-95% of the sum of the van der Waal radii are common though others could be used. If all conformations of the new amino acid have close contacts, the amino acid substitution is rejected. A conformation with no close contacts which can be matched to a preferred amino acid conformation as defined by Ponder, J.W., and Richards, F.M. (1987) J. Mol. Biol. 193:775-791, is most highly desirable. In step (6) the amino acid identified for change to the corresponding amino acid in the same position in the reference protein is changed by site-directed mutagenesis of the gene which expresses the target protein by the methods disclosed above.

The second method comprises the steps of:

- (1) generating a probe-accessible surface of said target protein by probing the three dimensional coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal cavities;
- (2) aligning said three dimensional coordinates of said target protein and a reference protein by moving the three dimensional coordinates of said reference protein into the coordinate frame of said target protein;
- (3) identifying an amino acid in said reference protein whose side chain lies outside said solvent-accessible surface of said protein or inside said internal cavities of said target protein;
- (4) identifying the amino acid in said target protein which occupies the equivalent position as

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said amino acid in said reference protein; (5) determining if said amino acid in said target protein can be changed without creating unacceptable steric effects; (6) replacing the amino acid in said target protein with the corresponding amino acid in the equivalent position in said reference protein by site-directed mutagenesis of the gene which expresses said target protein.

The first three steps of this method are the same as steps (1), (2), and (3) of the second method for the second computer based method for identifying the sites which affect the stability of a protein.

In step (4) the amino acid in the target protein which occupies the equivalent position as the amino acid in the reference protein is identified. Equivalency is determined from the primary sequence alignment and three dimensional structure alignment described above. Given two protein structures, a target and a reference structure, which have been aligned, equivalent amino acids are defined as pairs of amino acids, one from the target and one from the reference protein, which may differ in identity but occupy close to the same position in the secondary and tertiary structure of the two proteins.

The following examples are meant to illustrate but not to limit the invention.

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Example 1**Identification of Sites in BLAP for mutagenesis**

The structure of BLAP was obtained by X-ray crystallography and solved to 1.4 Å. The atomic coordinates are shown in Figure 1. Water molecules were removed from the structure and the protein coordinates were used to generate a probe-accessible surface using a computer program QUANTATM (version 3.0). This program can be used to calculate a probe-interaction map. The coordinates of BLAP were read into the computer and the following parameters were set in order to perform the probe interaction grid calculation. A Van der Waal calculation was requested with a "proton" probe (radius of 0.9 Å) with a charge of 0.0. The box dimensions were set to 50 Å with a grid size of 1 Å centered on the α -carbon of residue 219. The maximum energy was set to 500 and the minimum to -100. This means that energy values which exceed 500 will be set to 500. An energy value will exceed 500 when the probe is very close to an atom in the protein. The calculations were performed on a Silicon Graphics Inc. (2105 Landings Drive, Suite 2105, Mountain View, CA 94043) 4D/220 PowerIrisTM workstation. QUANTATM was used to visualize the probe-accessible surface. The map was contoured at 50 kcal/mol but this value depends on the particular constants in use and the method used to generate the probe accessible surface. The map was displayed simultaneously with the structure of BLAP and amino acid side chains which defined the boundaries of the internal cavities were identified visually.

One such amino acid was threonine-69. This side chain is completely buried with only 2% of its surface being solvent accessible. The hydroxyl group of the side chain defined part of the border of two internal cavities. These particular cavities are occupied by water molecules 278 on one side, and 280 on the other. Mutating this amino acid to valine represents a conservative change which increases the hydrophobicity of the side chain while having little

effect on size and shape. Using computer modeling, it was determined that mutating threonine-69 to valine would not create any close contacts with other protein atoms or significantly perturb the structure if the valine occupies the same position as the hydroxyl of threonine-69 in the wild type protein. An oligonucleotide was synthesized which carried a mutation of the codon for threonine-69 to valine (T69V). This oligonucleotide was used to create a site directed mutation in the BLAP gene which was subcloned into a *Bacillus* vector and expressed in *B. subtilis* DB104 (See Examples 4 and 5). Strains were identified which were expressing the mutant protease and several shake flasks were prepared to produce the mutant protein (See Example 5). The mutant protease was purified from the shake flask media and characterized for surfactant and temperature stability (See Examples 7, 10, and 11).

The mutation T69V resulted in a 340% increase in the half-life of the protease at 50°C, from 12 minutes to 41 minutes (See Table 3).

Example 2

Identification of Sites in BLAP for mutagenesis based on other proteases.

(A) Comparison to subtilisin Carlsberg.

The three dimensional coordinates of subtilisin Carlsberg (1CSE) were obtained from the Brookhaven Protein Database (Bernstein, F.C., et al. (1977) J. Mol. Biol. 112:535-542). The protease structures were aligned using the molecular modeling program QUANTATM. The BLAP coordinates were held fixed. The α -carbons of residues 1 to 32 of BLAP were matched to residues 1 to 32 of 1CSE, respectively; residues 40 to 60 of BLAP to residues 41 to 61 of 1CSE; residues 80 to 155 of BLAP to residues 82 to 157 of 1CSE; residues 170 to 269 of BLAP to residues 176 to 275 of 1CSE. The BLAP structure was held fixed, and the 1CSE structure was rotated and translated such that the r.m.s. deviation between the α -carbons of matched residues was minimized. The translation vector

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(-10.68738, 31.28904, -5.32134) and the rotation matrix
(0.17406 -0.65535 0.73500
-0.42119 -0.72422 -0.54599
0.89011 -0.21454 -0.40209)

5 were applied to the coordinates of 1CSE and the transformed
coordinates were saved (henceforth, the transformed 1CSE
structure). The final r.m.s. deviation between the matched
229 α -carbon pairs was 0.872 Å.

10 The probe-accessible surface map calculated in
Example 1 was used to color the atoms in the transformed
1CSE structure. The entire map, which consists of three
dimensional grid of (x, y, z) coordinates in space and an
energy value at each position, was read into computer
15 memory along with the protein coordinates (the transformed
1CSE structure). The energy value at each atom position
was approximated by interpolating from the energy values of
the surrounding eight nearest grid points in the probe map.
The protein atoms were colored on the basis of this
interpolated energy value. The protein was displayed using
20 QUANTA™ and atoms were displayed in different colors
depending on their interpolated energy value. For example,
if the energy were greater than 400 the atoms were dark
blue; between 300 and 400, light blue; 200 and 300, green;
200 to 100 yellow; and between -100 and 100, red. Visual
25 inspection of such a display allowed identification of side
chains which penetrated the solvent accessible surface or
internal cavities.

One such amino acid was methionine-199 (1CSE
numbering) in subtilisin Carlsberg. The amino acid was
30 identified by visual inspection of the transformed 1CSE
structure (as described above). Below, the coordinates of
residue 199 from the transformed 1CSE structure are shown
in the Brookhaven Protein Data Bank file format along with
the interpolated energy values.

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Coordinates of Methionine-199
from the 1.2 Å structure of subtilisin Carlsberg.

5	ATOM	1364	N	MET	199	22.392	40.705	32.311	1.0	500.00
	ATOM	1365	CA	MET	199	21.675	40.581	31.054	1.0	500.00
	ATOM	1366	C	MET	199	22.438	39.677	30.103	1.0	500.00
	ATOM	1367	O	MET	199	23.689	39.601	30.254	1.0	500.00
	ATOM	1368	CB	MET	199	21.621	41.991	30.511	1.0	500.00
10	ATOM	1369	CG	MET	199	20.868	42.994	31.426	1.0	500.00
	ATOM	1370	SD	MET	199	19.150	42.631	31.891	1.0	211.58
	ATOM	1371	CE	MET	199	18.273	43.395	30.493	1.0	41.68

Column 1 is the record type; column 2 is the atom number; column 3 is the atom name; column 4 is the residue name; column 5 is the residue number; columns 6, 7 & 8 are the x, y, z coordinates of the atom, respectively; column 9 is the occupancy; column 10 is normally the temperature factor but this has been replaced with the interpolated energy value. Note that a value of 500 in this column means that the atom is nearly completely within the van der Waal surface of the BLAP molecule. When the probe map was calculated (see Example 1), energy values greater than 500 were set to 500. As can be seen, atoms 1370 and 1371 have significantly lower energy values (column 10). The end of this methionine residue extends into an internal cavity in the BLAP molecule.

This residue is equivalent in secondary and tertiary structure to valine-193 in BLAP. Using computer modeling, valine-193 in BLAP was changed to methionine. The x values for the new methionine side chain in BLAP were taken from the subtilisin BPN' structure. In this conformation, the new side chain had no close contacts except for the ϵ -carbon of the methionine which contacted a crystallographic water in the BLAP structure.

An oligonucleotide was synthesized which mutated the codon for valine-193 to methionine (V193M) in the BLAP gene. This oligonucleotide was used to create a site directed mutation in the BLAP gene which was subcloned into a *Bacillus* vector and expressed in *B. subtilis* DB104 (See Examples 3, 4, and 5). Strains were identified which were expressing the mutant protease and several shake flasks were prepared to produce the mutant protein (See Example

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5). The mutant protease was purified from the shake flask media and characterized for temperature and surfactant stability (See Examples 6, 7, 10, and 11).

The mutation V193M resulted in a 350% increase in the half-life of the protease at 50°C, from 12 minutes to 42 minutes (See Table 3).

(B) Comparison to Thermitase.

The three-dimensional coordinates of thermitase (1TEC) were obtained from the Brookhaven Protein Database (Bernstein, F.C., et al. (1977) J. Mol. Biol. 112:535-542). The structures of BLAP and 1TEC were aligned using the molecular modeling program QUANTATM by matching equivalent α -carbons as listed below.

Matched α -carbons between BLAP and Thermitase (1TEC)	
<u>BLAP</u>	<u>1TEC</u>
5-20	12-27
23-34	29-41
43-72	52-81
75-227	85-237
232-256	240-264

The BLAP structure was held fixed and the 1TEC structure was rotated and translated such that the r.m.s. deviation between the α -carbons of matched residues was minimized. The translation vector (14.92521, 33.43270, 40.92134) and the rotation matrix

$$\begin{pmatrix} 0.79048 & -0.20395 & -0.57753 \\ -0.01688 & 0.93532 & -0.35340 \\ 0.61225 & 0.28911 & 0.73591 \end{pmatrix}$$

were applied to the coordinates of 1TEC and the transformed coordinates were saved (henceforth, the transformed 1TEC structure). The final r.m.s. deviation between the matched 236 α -carbon pairs was 1.384 Å.

The probe-accessible surface map was used to color the atoms in the transformed 1TEC structure. The entire probe map was read into computer memory along with the coordinates of the transformed 1TEC structure. The energy value at each atomic position was interpolated from the

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energy values of the eight surrounding grid points in the probe map. The protein was displayed using QUANTATM and atoms were displayed in different colors as a function of their interpolated energy value. For example, if the energy were greater than 400 the atoms were dark blue; between 300 and 400, light blue; 200 and 300, green; 200 to 100 yellow; and between -100 and 100, red. Visual inspection of such a display allowed identification of side chains which penetrated the solvent accessible surface or internal cavities.

One such amino acid was tyrosine-149 (1TEC numbering) in thermitase. The amino acid was identified by visual inspection of the transformed 1TEC structure. Below, the coordinates of residue 149 from the transformed 1TEC structure are shown in the Brookhaven Protein Data Bank file format along with the interpolated energy values.

Coordinates of Tyrosine-149

from the 2.0 Å structure of Thermitase.

20	ATOM	1052	N	TYR	149	19.783	23.026	47.326	1.0	500.00
	ATOM	1053	CA	TYR	149	20.372	21.668	47.275	1.0	500.00
	ATOM	1054	C	TYR	149	21.456	21.557	46.165	1.0	500.00
	ATOM	1055	O	TYR	149	22.619	21.330	46.486	1.0	500.00
	ATOM	1056	CB	TYR	149	19.282	20.595	47.169	1.0	500.00
25	ATOM	1057	CG	TYR	149	19.859	19.183	46.935	1.0	227.30
	ATOM	1058	CD1	TYR	149	20.262	18.427	48.038	1.0	79.13
	ATOM	1059	CD2	TYR	149	20.014	18.722	45.608	1.0	275.01
	ATOM	1060	CE1	TYR	149	20.762	17.146	47.807	1.0	10.99
	ATOM	1061	CE2	TYR	149	20.531	17.425	45.371	1.0	500.00
30	ATOM	1062	CZ	TYR	149	20.860	16.649	46.488	1.0	131.28
	ATOM	1063	OH	TYR	149	21.165	15.337	46.282	1.0	147.29

Column 10 is normally the temperature factor but this has been replaced with the interpolated energy value. As can be seen, the phenyl ring of the tyrosine side chain has significantly lower energy values (column 10 of atoms CG, CD1, CD2, CE1, CE2 and CZ).

This residue is equivalent in secondary and tertiary structure to serine-139 in BLAP. Using computer modeling, serine-139 in BLAP was changed to tyrosine. The χ values for the new tyrosine side chain in BLAP were taken from the thermitase structure. In this conformation, the new side chain had no close contacts that could not be alleviated by small changes (less than 5°) of the χ values. The modeled

tyrosine side chain in BLAP fits neatly into a crevice on the surface of the BLAP protein between two surface helices.

5 An oligonucleotide was synthesized which mutated the codon for serine-139 to tyrosine (S139Y) in the BLAP gene. This oligonucleotide was used to create a site directed mutation in the BLAP gene which was subcloned into a *Bacillus* vector and expressed in *B. subtilis* DB104 (See Examples 3, 4, and 5). Strains were identified which expressed the mutant protease and several shake flasks were prepared to produce the mutant protein (See Example 5). The mutant protease was purified from the shake flask culture and characterized for temperature and surfactant stability (See Examples 6, 7, 10, and 11).

10 The mutation S139Y resulted in a 216% increase in the half-life of the protease at 50°C, from 12 minutes to 26 minutes (See Table 3).

Example 3

Site Directed Mutagenesis of the BLAP gene

20 This mutagenesis procedure was first described by Stanissens, P., et al. (1989) *Nucleic Acids Res.* 17:4441-4454. While this is the preferred method, many other methods could be used to introduce oligonucleotide site-directed mutations, particularly those which use single stranded DNA. For example, the method of Kunkel (Kunkel, T.A. (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492) has also been used.

25 A synthetic oligonucleotide was synthesized which mutates the codon of threonine-69 to the codon for valine. The mutagenic oligonucleotide was annealed to a gapped duplex DNA which carries the BLAP gene on a segment of single stranded (ss) DNA. The gapped duplex (gd) was formed by denaturing linear DNA's from pMc13C and pMa5-8 followed by re-annealing. The mutagenic oligonucleotide annealed to homologous ss BLAP DNA within the gap and the remaining gap was filled in by a DNA polymerase and ligated using T4 DNA ligase. Subsequent treatment of the product

30

35

of the fill-in reaction with ExoIII removed any species with only partially filled gaps.

The product of the fill-in reaction was then transformed into a repair deficient *E. coli* strain such as WK6mutS. Plasmid DNA from the recombinant *E. coli* WK6mutS was prepared and transformed in a low plasmid/recipient ratio into a suppressor deficient strain of *E. coli* such as WK6. Ampicillin resistant transformants were selected and plasmid DNA of several candidates was purified and checked for the presence of the mutation.

The mutant BLAP protease was expressed by transferring the mutant BLAP genes from their particular *E. coli* pMa13C derivative vector into a plasmid vector which can replicate in *Bacillus* such as pH70 or pC51. In the following example, the plasmids pC51 and pH70 can be used interchangeably with the exception that plasmid pH70 encodes resistance to kanamycin while plasmid pC51 encodes resistance to tetracycline. The mutant BLAP gene was separated from the pMa13C plasmids by digestion with the restriction endonucleases *Ava*I and *Sst*I and then ligated with an *Ava*I-*Sst*I cut fragment of plasmid pH70 that includes the regions necessary for kanamycin resistance and for replication in *Bacillus*. The pH70 *Ava*I-*Sst*I fragment was purified by high pressure liquid chromatography (HPLC). After ligation the mutant BLAP plasmids were transformed into *B. subtilis* DB104, a strain that has been engineered to inactivate its own genes encoding the major alkaline and neutral proteases. *B. subtilis* DB104 transformed by these plasmids were grown on a nutrient-skim milk agar in the presence of the antibiotic kanamycin. Clones that manufactured mutant protease were identified by the formation of clear zones of hydrolysis in the skim milk. Plasmid DNA was purified from these clones to verify that the protease-producing clones carried the a plasmid-borne BLAP gene with the desired mutation. The plasmid DNA was purified away from cell protein and chromosomal DNA by SDS-salt precipitation followed by chromatography over a Qiagen

ion-exchange column (Qiagen Corporation). *Ava*I-*Sst*I digested plasmid DNAs from different clones were compared with *Ava*I/*Sst*I-digested derivatives of plasmid pH70 known to carry an intact BLAP gene. Plasmid digests were compared by agarose gel electrophoresis to identify plasmids that have the proper-sized *Ava*I/*Sst*I DNA fragments. Selected plasmid DNAs were then sequenced across the region of the particular BLAP mutation to confirm that the mutation was present. One or more clones of each BLAP mutation were stored frozen in 15% glycerol at -70°C and also cultivated in shake flasks (Examples 4 and 5) to manufacture mutant protease for characterization.

Example 4

Production of Proteases

Each strain of *B. subtilis* DB104 that carried a plasmid with one of the mutant BLAP genes was cultivated in shake flasks to make the mutant protease. Strains were grown in 50 ml precultures of (Difco) Luria Broth (LB) with the antibiotic kanamycin for pH70 derived clones or tetracycline for pC51 derived clones at 37°C and 280 rpm in a New Brunswick Series 25 Incubator Shaker. After 7 to 8 hours of incubation 2.5 or 5.0 ml of the preculture was transferred to 50 or 100 ml of MLBSP medium (Table 5), respectively, with either 20 µg/ml of kanamycin, or 15 µg/ml of tetracycline in 500 ml (Bellco) baffled shake flasks for growth and eventual production of the protease. These main shake flask cultures were incubated at 240 rpm and 37°C for 64 hours before the culture broths were treated to remove intact cells and cellular debris, and to reduce the pH to 5.8 before they were concentrated. The protease production of each culture was monitored by electrophoresis of culture supernatants with reverse polarity on 12.5% homogenous polyacrylamide gels with the Pharmacia PhastSystem.

Example 5

Production of Mutant Proteases in Shake Flasks

A hot loop was used to streak each mutant strain from a frozen cryovial culture onto an LB-skin milk agar containing either 20 $\mu\text{g/ml}$ of kanamycin or 15 $\mu\text{g/ml}$ of tetracycline. The plates were incubated at 37°C for 20 to 24 hours. A single, isolated colony producing a good zone of hydrolysis of the skim milk was picked into a 250 ml Erlenmeyer flask containing about 50 ml Luria Broth (LB) which contained either 20 $\mu\text{g/ml}$ kanamycin or 15 $\mu\text{g/ml}$ of tetracycline. The broth was incubated in a New Brunswick Series 25 Incubator Shaker at 37°C with shaking at 280 rpm for 7 to 8 hours. Either 2.5 ml of the turbid preculture was transferred into 50 ml of MLBSP containing either 20 $\mu\text{g/ml}$ kanamycin or 15 $\mu\text{g/ml}$ of tetracycline in each of four baffled 500 ml flasks, or 5 ml of preculture was used as an inoculum for 100 ml of MLBSP broth with antibiotic contained in each of two 500 ml baffled flasks (a 5% v/v transfer). All flasks were incubated at 240 rpm and 37°C for 64 hours. After 64 hours of incubation the set of flasks for each culture was consolidated, transferred to 50 ml centrifuge tubes, and centrifuged at 20,000 g_{av} for 15 minutes at 4°C. The broth was filtered through Miracloth (Calbiochem Corp. #475855) into 400 ml beakers chilled on ice. The broth was slowly stirred on ice for 30 minutes before the broth pH was reduced to 5.8 by the slow addition of glacial acetic acid. More fine debris were removed by centrifugation again at 20,000 g_{av} and the broth was filtered through Miracloth into graduated cylinders to measure the volume. Two sets of 1 ml samples were made for PhastSystem gels and activity assays. The broth was stored on ice until the protease could be purified. The MLBSP media used for the production of BLAP in shake flask cultures is described in Table 5.

TABLE 5
COMPOSITION OF MLBSP MEDIUM

5	Component	Quantity (for 1 liter of media)
10	deionized water	750 ml
	Difco Casitone	10 gm
	Difco Tryptone	20 gm
15	Difco Yeast Extract	10 gm
	NaCl	5 gm
20	Sodium Succinate	27 gm

25 The media was adjusted to pH of 7.2 by addition of NaOH, the volume adjusted to 815 ml with water and autoclaved 15 minutes at 121°C at 15 lbs/in². The media was cooled before adding the sterile stock solutions described in Appendix 1, while stirring.

APPENDIX 1 (additions to MLBSP broth)

30	Component	Quantity (for 1 L of media)
35	MgSO ₄ ·7H ₂ O (100 mg/ml stock, autoclaved)	1.0 ml
	CaCl ₂ ·2H ₂ O (30 mg/ml stock, autoclaved)	2.5 ml
	FeSO ₄ ·7H ₂ O (1mM stock, filter sterilized)	0.5 ml
	MnCl ₂ ·4H ₂ O (1mM stock, autoclaved)	0.5 ml
	Glucose (25% (w/v) stock, autoclaved)	80.0 ml
40	PIPES Buffer ¹ (pH 7.2, 1 M stock, autoclaved)	50.0 ml
	KPO ₄ Buffer ² (1.5 M stock, autoclaved)	50.0 ml

1 Piperazine-N,N'-bis(2-ethane sulfonic acid).

45 2 A sufficient amount of 1.5 M dibasic phosphate (K₂HPO₄) was added to 200 ml of 1.5 M monobasic phosphate (KH₂PO₄) to adjust the pH to 6.0 using a Beckman PHI44 pH meter equipped with a Beckman combination electrode (#3952C). The final pH was adjusted to 7.0 with 4 M KOH.

50 Either kanamycin or tetracycline antibiotic stock solutions were added to the media just before use to a final concentration of 20 µg/ml and 15 µg/ml respectively.

Example 6**Purification of BLAP**

5 Fermentation broth of transformed *B. subtilis* DB104, while still in the fermenter, was adjusted to pH 5.8 with 4 N H₂SO₄. The broth was collected and cooled to 4°C. If not mentioned otherwise, all subsequent steps were performed on ice or at 4°C. An aliquot of the broth material was clarified by centrifugation at 15,000 x g_{av.} for 60 min. Floating lipid material was removed by aspiration, and the supernatant filtered through Miracloth. 10 The dark brown solution was placed in dialysis tubing (Spectrapor; #1, 6 to 8 kilodalton (kDa) molecular-weight-cut-off, 1.7 ml/cm) and dialyzed for 16 hours in 20 mM 2-(N-morpholino)ethanesulfonic acid (MES) containing 1 mM CaCl₂, adjusted with NaOH to pH 5.8 ('MES buffer'). The dialysate was clarified by centrifugation (20,000 x g_{av.} for 10 min) and the pH of the solution was adjusted to 7.8 with 2 N NaOH. The enzyme solution containing approximately 0.9 g of protein in 1.2 liter was loaded at a flow rate of 20 150 ml/hour onto a column of S-Sepharose Fast Flow (SSFF, Pharmacia; 25 mm diameter, 260 mm long) previously equilibrated with 20 mM N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) [HEPES], containing 1 mM CaCl₂, adjusted with NaOH to pH 7.8 ('HEPES buffer'). After the application of the enzyme solution the column was washed 25 with 2 column volumes (250 ml) of HEPES buffer and then developed at a flow rate of 140 ml/hour with a gradient of 0 to 0.25 M NaCl in 600 ml of HEPES buffer. The gradient eluate was fractionated into 5.2-ml aliquots which were collected into tubes containing 2 ml of 100 mM MES/Na⁺, pH 5.8. The enzyme eluted between 0.12 and 0.15 M NaCl. Fractions containing the enzyme were pooled and protein was precipitated with ammonium sulfate at 52% of saturation. Solid salt (0.33 g per ml of solution) was added slowly 30 with stirring over a period of 15 min, and stirring was continued for another 15 min. The precipitate was collected by centrifugation, the pellet was dissolved in 35

MES buffer and the protein concentration in the solution was adjusted to 5 to 7 mg/ml. Following dialysis for 16 hours in MES buffer the solution was clarified by centrifugation and the pH of the supernatant was adjusted to 7.2. The protease was purified further by a second cation exchange separation on SSFF. All steps of this procedure were the same as above except that the pH of the HEPES buffer was 7.2 and that the NaCl gradient was from 0 to 0.25 M in 600 ml of HEPES buffer. Protein in pooled fractions was precipitated as above with ammonium sulfate and the enzyme was stored as ammonium sulfate precipitate at -70°C. Prior to use the ammonium sulfate precipitate of the enzyme was dissolved in an appropriate buffer, typically MES buffer, at the desired protein concentration, and dialyzed overnight in the buffer of choice.

Example 7

Purification of BLAP Mutants

Fermentation broth from shake flasks, on average 180 ml, was collected and clarified by centrifugation at 20,000 x g_{av} . for 15 min. The supernatant was placed, with stirring, on ice and after 30 min the pH of the solution was adjusted to 5.8 with glacial acetic acid. If not mentioned otherwise, all subsequent steps were performed on ice or at 4°C. The solution was clarified again by centrifugation (20,000 x g_{av} . for 15 min) and was concentrated approximately 4-fold by ultrafiltration (Amicon; YM30 membrane). The dark brown solution was placed in dialysis tubing (Spectrapor; #1, 6 to 8 KDa molecular-weight-cut-off, 1.7 ml/cm) and dialyzed for 16 hours in 20 mM HEPES/Na⁺, pH 7.8, containing 1 mM CaCl₂ ('HEPES buffer'). The dialysate was clarified by centrifugation (20,000 x g_{av} . for 10 min) and the pH of the solution, if necessary, was adjusted to 7.8 with 2 N NaOH. The enzyme solution was loaded at a flow rate of 60 ml/hour onto a column of SSFF (15 mm diameter, 75 mm long), previously equilibrated with HEPES buffer. When all colored by-products were eluted, the column was washed with

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50 ml of HEPES buffer. Then, the enzyme was eluted with 0.25 M NaCl in HEPES buffer. Fractions of 1.2 ml were collected into tubes containing 0.5 ml of 100 mM MES/Na⁺, pH 5.8. Protein content in fractions was monitored either by a UV detector set at 280 nm or by protein assay as described below. Pooled fractions containing protease protein were placed on ice and protein was precipitated with a 5 to 8-fold volume excess of acetone at -20°C. The protein was allowed to precipitate for 6 min, the mixture was centrifuged for 4 min at 6,600 x g_{av.}, the supernatant was discarded, the pellet was briefly exposed to vacuum (water aspirator) to remove most of the acetone, and the pellet was dissolved in 20 mM MES/Na⁺, pH 5.8, to give an approximate protein concentration of 30 mg/ml. Prior to any assays, the solution was centrifuged in an Eppendorf centrifuge for 3 min at full speed (13,000 x g_{max.}).

Example 8

Protein Determination

Protein was determined by a modified biuret method (Gornall, A.G., et al. (1948) J. Biol. Chem. 177:751-766). The protein in a total volume of 500 µl was mixed with 500 µl of biuret reagent and incubated for 10 min at 50°C. The solution was briefly chilled and its absorbance was measured at 540 nm. Typically, a reagent blank and three different protein aliquots in duplicates were measured and the recorded optical densities analyzed by linear regression. Bovine serum albumin (BSA, crystalline; Calbiochem) was used as protein standard. With purified BLAP protein the usefulness of BSA as protein standard in the biuret assay was confirmed. A BLAP sample was exhaustively dialyzed in 1 mM sodium phosphate, pH 5.8, and subsequently lyophilized. A sample of the solid material was weighed, dissolved in 1 mM sodium phosphate, pH 5.8, and used to generate a standard curve for the biuret assay. From the actual difference in phosphate content (Black, M.J., and Jones, M.E. (1983) Anal. Biochem. 135:233-238) of the final protein solution and the nominally 1 mM sodium

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phosphate solution used to dissolve the protein, the contribution of phosphate to the weight of solid BLAP was estimated and used to correct the standard curve.

Example 9

Protease Assays

Two different protease assays were used. With the HPE method protease activity was established at a single concentration of casein (prepared according to Hammarsten; Merck, #2242) as substrate. In the AAPF-pNA assay initial rates of succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanyl-p-nitroanilide (AAPF-pNA; Bachem) supported catalysis were used to determine the kinetic parameters K_m , k_{cat} , and k_{cat}/K_m .

A. HPE Method.

Culture supernatants or solutions of purified proteases were diluted with chilled buffer (10 mM MES/Na⁺, pH 5.8) to give three different solutions with a protein concentration ratio of 1:3:5. The substrate solution contained 9.6 mg/ml casein, 24 mM Tris, and 0.4% (w/v) sodium tripolyphosphate, dissolved in synthetic tap water (STW; 0.029% (w/v) CaCl₂·2H₂O, 0.014% (w/v) MgCl₂·6H₂O, and 0.021% (w/v) NaHCO₃ in deionized water) adjusted to pH 8.5 at 50°C, prepared as follows. With stirring for 10 min, 6 g of casein was dissolved in 350 ml of STW. To this, 50 ml of 0.3 M Tris in STW was added and stirring was continued for another 10 min. This solution was heated to 70°C, then allowed to cool slowly. At 50°C, the pH was adjusted to 8.5 with 0.1 N NaOH. When the solution reached room temperature, the volume was adjusted to 500 ml with STW, followed by the addition of 125 ml of 2% (w/v) pentasodium tripolyphosphate in STW, pH 8.5 (adjusted with 3 N HCl). The protease assay was started by adding 50 µl of protease solution to 750 µl of substrate solution placed in a 2.2 ml Eppendorf container preincubated for 10 min at 50°C. After 15 min, the reaction was terminated by the addition of 600 µl of trichloroacetic reagent (0.44 M trichloroacetic acid, 0.22 M sodium acetate in 3% (v/v)

glacial acetic acid). The mixture was placed on ice for 15 min, the precipitated protein removed by centrifugation for 8 min (at $13,000 \times g_{max.}$) and a 900 μ l aliquot of the supernatant was mixed with 600 μ l of 2 N NaOH. The absorbance at 290 nm of this solution was recorded. Each dilution was assayed in duplicates and the data points for three different dilutions from one enzyme sample was analyzed by linear regression. A slope of 1 in this assay corresponds to 80 HPE units in the least diluted sample. In case of strongly colored culture supernatants with measurable quantities of UV absorbing material carried over by the diluted protease aliquot into the assay cuvette a control curve was constructed whose slope was subtracted from the slope of the protease assay before final HPE units were calculated.

B. AAPF-pNA Assay

Protease samples were diluted with 50% (v/v) 1,2-propanediol in 100 mM Tris, adjusted with 2 N HCl to pH 8.6 at 25°C ('Tris-propanediol buffer'), in which they were stable for at least 6 h at room temperature. A stock solution of 160 mM AAPF-pNA was prepared in dimethylsulfoxide dried with a molecular sieve (Aldrich; 4 Å, 4-8 mesh) for at least 24 h prior to use. Fixed point assays were performed at 25°C with 1.6 mM AAPF-pNA in 100 mM Tris, adjusted with 2 N HCl to pH 8.6 at 25°C, in a total volume of 1.020 ml. The substrate was added to the assay buffer 1 min prior to the assay initiation and the reaction was started by addition of enzyme at a final concentration of 20 ng to 1.3 μ g of protein per ml (0.75 to 48.5 nM enzyme) depending on specific activity. Release of p-nitroanilide was monitored at 410 nm, and a molar extinction coefficient of $8,480 \text{ M}^{-1}\text{cm}^{-1}$ was used to calculate amount and concentration of product formed (DelMar, E.G., et al. (1979) Anal. Biochem. 99:316-320). Kinetic parameters were calculated from a velocity vs. substrate concentration plot constructed from initial rates measured once each at 12 different AAPF-pNA concentrations

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ranging from 0.16 to 3.2 mM. Data were fitted to a hyperbolic curve and proportionally weighted using the program ENZFITTER (Leatherbarrow, R.J. (1987) ENZFITTER, Biosoft, Cambridge, UK). A nominal molecular weight of 26.8 kDa was used in all calculations that required the interconversion of protein concentration and molarity of protease enzyme.

Example 10

Temperature Stability of Purified Proteases

Stability of protease proteins was evaluated under two different conditions: (a) 100 mM glycine/Na⁺, pH 10 at 60°C, and (b) 100 mM glycine/Na⁺, pH 11 at 50°C. At t = 0 min, the protein was diluted to approximately 0.25 mg/ml into incubation buffer maintained at the desired temperature. Periodically, an aliquot was removed from this incubation mixture and diluted into Tris-propanediol buffer chilled on ice. Residual protease activity was determined by the AAPF-pNA assay at a fixed AAPF-pNA concentration (1.6 mM). Stability is expressed as half-life ($t_{1/2}$) of activity determined from semi-logarithmic plots of residual activity as function of time. Each plot consisted of 6 data points with $t_{1/2}$ approximately in the center between experimental points.

Example 11

Resistance of Proteases to Sodium Dodecylsulfate (SDS)

SDS was selected as representative of surfactants in general. Resistance of proteases to SDS was evaluated under two different conditions: (a) 100 mM Tris adjusted with 2 N HCl to pH 8.6 at 50°C, containing 1% (w/v) SDS, and (b) 50 mM sodium carbonate, pH 10.5 at 50°C, containing 1% (w/v) SDS. Protease proteins were incubated at a final protein concentration of 0.25 mg/ml. Data were collected and evaluated as described above under Example 10.

Example 12

Polyacrylamide Gel Electrophoresis

Purity of protease samples was evaluated on 20% non-denaturing PhastSystem gels (Pharmacia) run with reversed

polarity. The same system was used to monitor the protease content of crude shake flask and fermentation broths. Buffer strips were prepared as described in Application File No. 300 (Pharmacia).

5 Molecular weight determinations were performed on 20% SDS PhastSystem gels, using the following markers: bovine serum albumin, 66 kDa; egg albumin, 45 kDa; glyceraldehyde-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20.1 kDa; 10 α -lactalbumin, 14.2 kDa (all from Sigma). Prior to SDS-PAGE, a protease sample was denatured with formic acid at a final concentration of 30 to 50% (v/v). Upon dilution of formic acid to 15% (v/v) protein was precipitated with trichloroacetic acid at a final concentration of 10% (v/v). 15 The collected pellet was washed once with water, then dissolved in 2% (w/v) SDS and heated for 2 min in a boiling waterbath. Gels were stained with Coomassie Brilliant Blue R-250 (Kodak).

DEPOSIT OF MICROORGANISMS

20 Living cultures of the following have been accepted for deposit under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the purposes of patent procedure by the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 on 25 May 8, 1991 (the accession number preceeds each deposit description): ATCC 68614 - *Bacillus licheniformis* ATCC 53926 strain which contains a tetracycline-resistance plasmid originally derived from *Bacillus* plasmid pBC16 which carries the ATCC 53926 alkaline protease-BLAP *Cla*I fusion gene, whose structural gene has the mutations S3T, 30 V4I, A188P, V193M, V199I; ATCC 68615 - *E. coli* WK6 which carries phasmid pMc13C, a chloramphenicol-resistant derivative of phasmid pMc5-8, that contains the ATCC 53926 alkaline protease- BLAP *Cla*I fusion gene and a 164 bp *Kpn*I 35 fragment carrying the ATCC 53926 alkaline protease gene's transcriptional terminator. The genotype of strain WK6 are Δ lac-proAB, galE, strA, mutS::Tn10/F'lacI^q, ZAM15,

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proA⁺B⁺ (Zell, R., and Fritz, H. -J. (1987) EMBO J. 6:1809-1815); ATCC 68616 - *E. coli* GM33 which carries plasmid pCB13C, an ampicillin-resistant derivative of Pharmacia plasmid vector pTZ19R (Pharmacia) that contains the ATCC 53926 alkaline protease-*Cla*I fusion gene. The GM33 strain's genotype is dam3 (dam-methylase minus (Marinus, M.G. and Morris, N.R. (1974) J. Mol. Biol. 85:309-322)); ATCC 68617 - *E. coli* WK6 which carries phasmid pMa5-8, an ampicillin-resistant mutagenesis vector described in Stanssens, P. et al. (1989) Nucleic Acids Research 17:4441-4454. The genotype of strain WK6 mutations are Alac-proAB, galE, strA, mutS::Tn10/F'lacI^q, ZAM15, proA⁺B⁺ (Zell, R., and Fritz, H. -J. (1987) EMBO J. 6:1809-1815); ATCC 68618 - an *E. coli* WK6 which carries phasmid pMc5-8, a chloramphenicol-resistant mutagenesis vector described in Stanssens, P., et al. (1989) Nucleic Acids Res. 17:4441-4454. The genotype of strain WK6 are Alac-proAB, galE, strA, mutS::Tn10/F'lacI^q, ZAM15, proA⁺B⁺ (Zell, R., and Fritz, H. -J. (1987) EMBO J. 6:1809-1815).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Christiansen, Teresa
Goddette, Dean W.
Ladin, Beth F.
Lau, Maria R.
Paech, Christian
Reynolds, Robert B.
Wilson, Charles R.
Yang, Shiow-Shong
- (ii) TITLE OF INVENTION: Third Generation Protease Mutants
- (iii) NUMBER OF SEQUENCES: 105
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Henkel Corporation
 - (B) STREET: 140 Germantown Pike, Suite 150
 - (C) CITY: Plymouth Meeting
 - (D) STATE: Pennsylvania
 - (E) COUNTRY: USA
 - (F) ZIP: 19462
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Drach, John E.
 - (B) REGISTRATION NUMBER: 32891
 - (C) REFERENCE/DOCKET NUMBER: M4922
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 215-832-2215
 - (B) TELEFAX: 215-941-6067

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 269 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

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- (ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843
(vii) IMMEDIATE SOURCE:
 (B) CLONE: S3T, V4I, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

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His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
180 185 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139Y, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Tyr Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 269 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S130T, S139Y, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

61

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Thr Ala Thr Leu Glu Gln Ala Val Asn Tyr Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

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Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110
 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125
 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190
 Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease
(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S3T, A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Gln Thr Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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65

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S157T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

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66

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Thr Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

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Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: *Bacillus lentus* DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

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Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235

240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 269 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: A188P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

70

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S3T, V4I, A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Met Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

SUBSTITUTE SHEET

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease
(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S104T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Thr Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: T69V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

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Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Val Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: V4I, A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Gln Ser Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

SUBSTITUTE SHEET

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
180 185 190

Met Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

SUBSTITUTE SHEET

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: A224V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

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Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: V4I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Gln Ser Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S3T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala Gln Thr Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S139Y

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

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Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Tyr Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

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Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: N242A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

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Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Ala His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 269 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S236T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

SUBSTITUTE SHEET

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Thr Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S36A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ala Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

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Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: H243A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

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Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn Ala Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

SUBSTITUTE SHEET

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: A101T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Thr Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 269 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Serine Protease
 - (B) STRAIN: Bacillus lentus DSM 5843
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: S236A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15
 His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ala Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E87R

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Arg Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: N114S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Ser Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A47W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Trp Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

SUBSTITUTE SHEET

102

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A120S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ser Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

SUBSTITUTE SHEET

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: T56V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Val Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A120V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Val Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

SUBSTITUTE SHEET

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: *Bacillus lentus* DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: G205V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Val Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S130A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ala Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

SUBSTITUTE SHEET

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S130T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Thr Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250

112

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A96I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ile
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

113

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 269 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S104T, S139Y, A224V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Thr Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Tyr Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S139A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ala Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S142T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Thr Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Thr Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: I102W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Trp Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

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Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A96N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Asn
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:
 (B) CLONE: N42F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Phe Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S142A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

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Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ala Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile .
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

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Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 269 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Serine Protease
 (B) STRAIN: Bacillus lentus DSM 5843
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: H118F
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
- Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15
- His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30
- Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45
- Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60
- His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80
- Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95
- Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

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Gly Asn Asn Gly Met Phe Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

SUBSTITUTE SHEET

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: N237A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Ala Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: N255P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

SUBSTITUTE SHEET

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Pro Leu
245 250 255

SUBSTITUTE SHEET

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: T141W, N237A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

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Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Trp Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Ala Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: Serine Protease
(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:
(B) CLONE: T268V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

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Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Val Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Serine Protease
- (B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

- (B) CLONE: K229W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

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Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Trp Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

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Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: T141W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

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Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Trp Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 269 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serine Protease

(B) STRAIN: Bacillus lentus DSM 5843

(vii) IMMEDIATE SOURCE:

(B) CLONE: wildtype

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asp Gly Arg Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Ser Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

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Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S3T, V4I, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GCGCAAACAA TCCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
 60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
 120

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TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACATTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 807 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (B) STRAIN: Bacillus lentus DSM 5483
- (vii) IMMEDIATE SOURCE:

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(B) CLONE: S3T, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GCGCAAACAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AACATTTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
(B) STRAIN: *Bacillus lentus* DSM 5483
- (vii) IMMEDIATE SOURCE:
(B) CLONE: V4I, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCGCAATCAA TCCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGTTTGGA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACATTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

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CTTGTC AATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139Y, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATTATGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACATTCAG
600

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AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S130T, S139Y, A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

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AATTTGAGTT TAGGAAGCCC TTCGCCAACA GCCACACTTG AGCAAGCTGT TAATTATGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACATTTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A188P, V193M, V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

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GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACATTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 807 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (B) STRAIN: *Bacillus lentus* DSM 5483
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: S3T, A188P, V193M

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GCGCAAACAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60
TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120
TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180
GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTAA ACAATTCGAT TGGCGTCTT
240
GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480
TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACGTGCAG
600
AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720
CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780
CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S157T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60
TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120
TTAAATATTC GTGGTGCGGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180
GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240
GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAC ATCAATCAGC
480
TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600
AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720
CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780
CTTGTCATG CAGAAGCGGC AACACGC
807

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(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

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151

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A188P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

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ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTGTCTG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S3T, V4I, A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GCGCAAACAA TCCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

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GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480
TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACGTGCAG
600
AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720
CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780
CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CGGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60
TTGACAGGTT CTGGTGTAAA AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120
TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180
GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240
GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480
TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTATGG CACCAGGGGT AAACGTGCAG
600
AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720
CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780
CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S104T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCAGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCA CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:67:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: T69V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

ACGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

GTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

CTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

AGGCATGGCA CGCATGTGGC CGGGGTTATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

AGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

CAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

ATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

CTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

ATCCGGCCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

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GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAL G CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: V4I, A188P, V193M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GCGCAATCAA TCCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

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TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CCCAGGGCTT GACATTATGG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A224V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

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WO 92/21760

PCT/US92/04306

GGCGTAGCGC CTAGTGC GGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG TTGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: V199I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGA
60

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TTGACAGGTT CTGGTGTAAA AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACATTCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

TGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: V4I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GCGCAATCAA TCCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

SUBSTITUTE SHEET

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (B) STRAIN: *Bacillus lentus* DSM 5483
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: S3T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GCAAACAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

GACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

AAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

CGTAGCGC CTAGTGC GGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

AATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

TTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

CACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

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CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139Y

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCAGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATTATGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

SUBSTITUTE SHEET

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: N242A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

SUBSTITUTE SHEET

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCGCACATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S236T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGACAAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

SUBSTITUTE SHEET

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S36A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTGCAAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 807 base pairs

SUBSTITUTE SHEET

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (B) STRAIN: Bacillus lentus DSM 5483
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: H243A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

SCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCCA TAACCGTGGA
60
ACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120
AATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180
SCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240
GTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
ATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
TTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
TCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480
CCGGCCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
AGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600
ACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

SUBSTITUTE SHEET

CGCAACGCAC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAA¹GC²GGA³
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A101T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTA¹AA AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGC²GGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

ACAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S236A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

SUBSTITUTE SHEET

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGGCAAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTC AATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: E87R

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

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TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGCG TCTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

SUBSTITUTE SHEET

(vii) IMMEDIATE SOURCE:
(B) CLONE: N114S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA GCAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 807 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

SUBSTITUTE SHEET

(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
(B) STRAIN: *Bacillus lentus* DSM 5483
- (vii) IMMEDIATE SOURCE:
(B) CLONE: A47W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

GACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

AAATATTC GTGGTGGCTG GAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

CGTAGCGC CTAGTGCAGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

AATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

TTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

TAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

TACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

TAAACATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

SUBSTITUTE SHEET

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 807 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A120S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTAGC
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

SUBSTITUTE SHEET

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: T56V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCGTTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCAGG ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

SUBSTITUTE SHEET

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A120V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

SUBSTITUTE SHEET

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGTT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

SUBSTITUTE SHEET

(B) CLONE: G205V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGTTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 807 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S130A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAA AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAGCA GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S130T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAACA GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

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AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGTCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A96I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

CGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

IGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

CGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

CGGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAATTGA CGGTAGAGGT
300

CAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

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AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S104T, S139Y, A224V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

CAATCAGCA CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

ATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATTATGCG
420

CTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

ATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGCAGGTG TTGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATGCAGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 807 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S142T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

CAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

ATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

CTACAAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

ATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S139T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATACAGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

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TTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: I102W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

GTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

CATGGAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

ATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: A96N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

CGGTAGCGC CTAGTGC GGA ACTATACGCT GTTAAAGTTT TAGGAAACGA CGGTAGAGGT
300

CAATCAGCT CGATTGCCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

ATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

CTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

ATCCGGCCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

CCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCTG CACCAGGGGT AAACGTGCAG
600

GCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

GCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTGTCAATG CAGAAGCGGC AACACGC
807

2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: N42F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

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GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCC TAACCGTGGG
60

TTGACAGGTT CTGGTGTAAA AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTATTTATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCC AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

SUBSTITUTE SHEET

WO 92/21760

PCT/US92/04306

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: S142A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240GGCGTAGCGC CTAGTGCAGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420ACTGCAAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: H118F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GTTTGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCT CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

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MTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

SCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

MTGTCAATG CAGAAGCGGC AACACGC
807

2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: N237A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

CGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

CAATATTC GTGGTGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

CGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

CGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

CAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

TTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

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TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCGC TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: N255P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

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196

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CGTAGCGC CTAGTGC GGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

AATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

TTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

CCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

AGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

CATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
60

CAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
20

ACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGCCATTGTA TGGAAGCGGA
80

CAATG CAGAAGCGGC AACACGC
807

INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 807 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

i) MOLECULE TYPE: DNA (genomic)

i) HYPOTHETICAL: NO

v) ANTI-SENSE: NO

) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

) IMMEDIATE SOURCE:

(B) CLONE: T141W, N237A

SEQUENCE DESCRIPTION: SEQ ID NO:102:

AG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TGGTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCGC TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:

(B) STRAIN: *Bacillus lentus* DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: T268V

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780CTTGTCAATG CAGAAGCGGC AGTTCGC
807

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 807 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(B) STRAIN: *Bacillus lentus* DSM 5483
(vii) IMMEDIATE SOURCE:
(B) CLONE: K229W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60
TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120
TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180
GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240
GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300
GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360
AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420
ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480
TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540
GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600
AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660
GTTGCAGGTG CAGCAGCCCT TGTTTGGCAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

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CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(B) STRAIN: Bacillus lentus DSM 5483

(vii) IMMEDIATE SOURCE:

(B) CLONE: T141W

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CGGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGAGGT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

TGGTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

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TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA ^{PCT/US92/04306} CAATCAATCC
540

GCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCTG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

GTTGCAGGTG CAGCAGCCCT TGTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGAAGCGGA
780

CTTGTCAATG CAGAAGCGGC AACACGC
807

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus lentus*
- (B) STRAIN: DSM 5483

(vii) IMMEDIATE SOURCE:

- (B) CLONE: wild type

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GCGCAATCAG TGCCATGGGG AATTAGCCGT GTGCAAGCCC CGGCTGCCCA TAACCGTGGA
60

TTGACAGGTT CTGGTGTAAG AGTTGCTGTC CTCGATACAG GTATTTCCAC TCATCCAGAC
120

TTAAATATTC GTGGTGGCGC TAGCTTTGTA CCAGGGGAAC CATCCACTCA AGATGGGAAT
180

GGGCATGGCA CGCATGTGGC CGGGACGATT GCTGCTTTAA ACAATTCGAT TGGCGTTCTT
240

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GGCGTAGCGC CTAGTGCGGA ACTATACGCT GTTAAAGTTT TAGGAGCCGA CGGTAGACTT
300

GCAATCAGCT CGATTGCCCA AGGGTTGGAA TGGGCAGGGA ACAATGGCAT GCACGTTGCT
360

AATTTGAGTT TAGGAAGCCC TTCGCCAAGT GCCACACTTG AGCAAGCTGT TAATAGCGCG
420

ACTTCTAGAG GCGTTCTTGT TGTAGCGGCA TCTGGGAATT CAGGTGCAAG CTCAATCAGC
480

TATCCGGCCC GTTATGCGAA CGCAATGGCA GTCGGAGCTA CTGACCAAAA CAACAACCGC
540

SCCAGCTTTT CACAGTATGG CGCAGGGCTT GACATTGTCG CACCAGGGGT AAACGTGCAG
600

AGCACATACC CAGGTTCAAC GTATGCCAGC TTAAACGGTA CATCGATGGC TACTCCTCAT
660

TTTGCAGGTG CAGCAGCCCT TGTTAAACAA AAGAACCCAT CTTGGTCCAA TGTACAAATC
720

CGCAACCATC TAAAGAATAC GGCAACGAGC TTAGGAAGCA CGAACTTGTA TGGAAGCGGA
780

TTTGTCAATG CAGAAGCGGC AACACGC
807

What is claimed is:

1. A mutant *Bacillus lentus* DSM 5483 protease derived by the replacement of at least one amino acid residue of the mature form of the *Bacillus lentus* DSM 5483 alkaline protease shown in SEQ ID NO:52 wherein said one amino acid residue which is selected from the group consisting of Ser3, Val4, Ser36, Asn42, Ala47, Thr56, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114, His118, Ala120, Ser130, Ser139, Thr141, Ser142, Ser157, Ala188, Val193, Val199, Gly205, Ala224, Lys229, Ser236, Asn237, Asn242, His243, Asn255, Thr268 is replaced with the amino acid residues listed in Table 2.

2. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine, and the serine residue at position 3 is substituted by threonine.

3. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

4. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

5. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted

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by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 139 is substituted by tyrosine.

5

6. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the serine residue at position 139 is substituted by tyrosine and the serine residue at position 130 is substituted by threonine.

10

7. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline.

15

8. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

20

9. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 157 is substituted by threonine.

25

10. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline.

30

11. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 188 is substituted by proline.

35

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12. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine and the serine residue at position 3 is substituted by threonine.

13. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 193 is substituted by methionine.

14. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 104 is substituted by threonine.

15. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the threonine residue at position 69 is substituted by valine.

16. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

17. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 224 is substituted by valine.

18. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 199 is substituted by isoleucine.

19. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the valine residue at position 4 is substituted by isoleucine.

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20. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 3 is substituted by threonine.

5 21. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 139 is substituted by tyrosine.

10 22. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 242 is substituted by alanine.

15 23. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 236 is substituted by threonine.

20 24. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 36 is substituted by alanine.

25 25. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the histidine residue at position 243 is substituted by alanine.

30 26. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 101 is substituted by threonine.

5 27. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 236 is substituted by alanine.

5 28. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the glutamic acid residue at position 87 is substituted by arginine.

29. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 114 is substituted by serine.

5 30. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 47 is substituted by tryptophan.

10 31. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 120 is substituted by serine.

15 32. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the threonine residue at position 56 is substituted by valine.

20 33. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 120 is substituted by valine.

34. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the glycine residue at position 205 is substituted by valine.

25 35. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 130 is substituted by alanine.

30 36. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 130 is substituted by threonine.

35 37. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 96 is substituted by isoleucine.

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38. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 224 is substituted by valine, the serine residue at position 104 is substituted by threonine, and the serine residue at position 139 is substituted by tyrosine.

39. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 139 is substituted by alanine.

40. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 142 is substituted by threonine.

41. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 139 is substituted by threonine.

42. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the isoleucine residue at position 102 is substituted by tryptophan.

43. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the alanine residue at position 96 is substituted by asparagine.

44. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 42 is substituted by phenylalanine.

45. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the serine residue at position 142 is substituted by alanine.

46. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the histidine residue at position 118 is substituted by phenylalanine.

47. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 237 is substituted by alanine.

5 48. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 255 is substituted by proline.

10 49. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the asparagine residue at position 237 is substituted by alanine and the threonine residue at position 141 is substituted by tryptophan.

15 50. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the threonine residue at position 268 is substituted by valine.

20 51. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the lysine residue at position 229 is substituted by tryptophan.

25 52. A mutant *Bacillus lentus* DSM 5483 protease of claim 1 wherein the threonine residue at position 141 is substituted by tryptophan.

30 53. A mutant gene which encodes for a mutant *Bacillus lentus* DSM 5483 protease comprising in the direction of transcription a promoter, ribosomal binding site, initiation codon and the major portion of the pre region of the *Bacillus licheniformis* ATCC 53926 alkaline protease gene operably linked to a portion of the pre region and all of the pro and mature regions of the *Bacillus lentus* DSM 5483 alkaline protease gene wherein one or more codons of said *Bacillus lentus* DSM 5483 alkaline protease gene are
35 altered to produce a mutant gene which encodes for a protease derived by the replacement of at least one amino acid residue of the mature form of the *Bacillus lentus* DSM

5483 alkaline protease wherein said one amino acid residue which is selected from the group consisting of Ser3, Val4, Ser36, Asn42, Ala47, Thr56, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114, His118, Ala120, Ser130, Ser139, Thr141, Ser142, Ser157, Ala188, Val193, Val199, Gly205, Ala224, Lys229, Ser236, Asn237, Asn242, His243, Asn255, Thr268 is replaced with the amino acid residues listed in Table 2.

54. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine, and the serine residue at position 3 is substituted by threonine.

55. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

56. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

57. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine

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residue at position 188 is substituted by proline, and the serine residue at position 139 is substituted by tyrosine.

5 58. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the serine residue at position 139 is substituted by tyrosine and the serine residue at position 130 is substituted by threonine.

15 59. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline.

20 60. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

25 61. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 157 is substituted by threonine.

30 62. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline.

35 63. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 188 is substituted by proline.

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64. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine and the serine residue at position 3 is substituted by threonine.

65. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine.

66. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 104 is substituted by threonine.

67. The mutant gene of claim 53 which encodes for said mutant protease wherein the threonine residue at position 69 is substituted by valine.

68. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

69. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 224 is substituted by valine.

70. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine.

71. The mutant gene of claim 53 which encodes for said mutant protease wherein the valine residue at position 4 is substituted by isoleucine.

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72. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 3 is substituted by threonine.

5 73. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 139 is substituted by tyrosine.

10 74. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 242 is substituted by alanine.

15 75. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 236 is substituted by threonine.

20 76. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 36 is substituted by alanine.

77. The mutant gene of claim 53 which encodes for said mutant protease wherein the histidine residue at position 243 is substituted by alanine.

25 78. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 101 is substituted by threonine.

30 79. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 236 is substituted by alanine.

35 80. The mutant gene of claim 53 which encodes for said mutant protease wherein the glutamic acid residue at position 87 is substituted by arginine.

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81. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 114 is substituted by serine.

5 82. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 47 is substituted by tryptophan.

10 83. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 120 is substituted by serine.

15 84. The mutant gene of claim 53 which encodes for said mutant protease wherein the threonine residue at position 56 is substituted by valine.

20 85. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 120 is substituted by valine.

86. The mutant gene of claim 53 which encodes for said mutant protease wherein the glycine residue at position 205 is substituted by valine.

25 87. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 130 is substituted by alanine.

0 88. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 130 is substituted by threonine.

89. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 96 is substituted by isoleucine.

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90. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 224 is substituted by valine, the serine residue at position 139 is substituted by tyrosine and the serine residue at position 104 is substituted by threonine.

91. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 139 is substituted by alanine.

92. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 142 is substituted by threonine.

93. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 139 is substituted by threonine.

94. The mutant gene of claim 53 which encodes for said mutant protease wherein the isoleucine residue at position 102 is substituted by tryptophan.

95. The mutant gene of claim 53 which encodes for said mutant protease wherein the alanine residue at position 96 is substituted by asparagine.

96. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 42 is substituted by phenylalanine.

97. The mutant gene of claim 53 which encodes for said mutant protease wherein the serine residue at position 142 is substituted by alanine.

98. The mutant gene of claim 53 which encodes for said mutant protease wherein the histidine residue at position 118 is substituted by phenylalanine.

99. The mutant gene of claim 53 which encodes for said mutant protease wherein the histidine residue at position 118 is substituted by phenylalanine.

99. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 237 is substituted by alanine.

5 100. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 255 is substituted by proline.

10 101. The mutant gene of claim 53 which encodes for said mutant protease wherein the asparagine residue at position 237 is substituted by alanine and the threonine residue at position 141 is substituted by tryptophan.

15 102. The mutant gene of claim 53 which encodes for said mutant protease wherein the threonine residue at position 268 is substituted by valine.

20 103. The mutant gene of claim 53 which encodes for said mutant protease wherein the lysine residue at position 229 is substituted by tryptophan.

25 104. The mutant gene of claim 53 which encodes for said mutant protease wherein the threonine residue at position 141 is substituted by tryptophan.

30 105. A hybrid plasmid capable of replication in *Bacillus* comprised of a gene which encodes for a mutant *Bacillus lentus* DSM 5483 protease comprising in the direction of transcription a promoter, ribosomal binding site, initiation codon and the major portion of the pre region of the *Bacillus licheniformis* ATCC 53926 alkaline protease gene operably linked to a portion of the pre region and all of the pro and mature regions of the *Bacillus lentus* DSM 5483 alkaline protease gene followed by a 164 bp DNA
35 fragment containing the transcription terminator from the ATCC 53926 alkaline protease gene wherein one or more codons of said *Bacillus lentus* DSM 5483 alkaline protease

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gene are altered to produce a mutant gene which encodes for a protease derived by the replacement of at least one amino acid residue of the mature form of the *Bacillus lentus* DSM 5483 alkaline protease wherein said one amino acid residue which is selected from the group consisting of Ser3, Val4, Ser36, Asn42, Ala47, Thr56, Thr69, Glu87, Ala96, Ala101, Ile102, Ser104, Asn114, His118, Ala120, Ser130, Ser139, Thr141, Ser142, Ser157, Ala188, Val193, Val199, Gly205, Ala224, Lys229, Ser236, Asn237, Asn242, His243, Asn255, Thr268 is replaced with the amino acid residues listed in Table 2.

106. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine, and the serine residue at position 3 is substituted by threonine.

107. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

108. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

109. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 139 is substituted by tyrosine.

110. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the serine residue at position 139 is substituted by tyrosine and the serine residue at position 130 is substituted by threonine.

111. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine, the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline.

112. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, and the serine residue at position 3 is substituted by threonine.

113. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 157 is substituted by threonine.

114. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine

residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline.

5 115. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 188 is substituted by proline.

10 116. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, the alanine residue at position 188 is substituted by proline, the valine residue at position 4 is substituted by isoleucine and the serine residue at position 3 is substituted by threonine.

15 117. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine.

20 118. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 104 is substituted by threonine.

25 119. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the threonine residue at position 69 is substituted by valine.

30 120. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 193 is substituted by methionine, and the alanine residue at position 188 is substituted by proline, and the valine residue at position 4 is substituted by isoleucine.

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121. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 224 is substituted by valine.

5 122. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 199 is substituted by isoleucine.

10 123. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the valine residue at position 4 is substituted by isoleucine.

15 124. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 3 is substituted by threonine.

20 125. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 139 is substituted by tyrosine.

25 126. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 242 is substituted by alanine.

30 127. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 236 is substituted by threonine.

35 128. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 36 is substituted by alanine.

40 129. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the histidine residue at position 243 is substituted by alanine.

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130. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 101 is substituted by threonine.

5 131. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 236 is substituted by alanine.

10 132. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the glutamic acid residue at position 87 is substituted by arginine.

15 133. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 114 is substituted by serine.

20 134. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 47 is substituted by tryptophan.

25 135. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 120 is substituted by serine.

136. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the threonine residue at position 56 is substituted by valine.

30 137. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 120 is substituted by valine.

35 138. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the glycine residue at position 205 is substituted by valine.

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139. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 130 is substituted by alanine.

5 140. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 130 is substituted by threonine.

10 141. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 96 is substituted by isoleucine.

15 142. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 224 is substituted by valine, the serine residue at position 139 is substituted by tyrosine and the serine residue at position 104 is substituted by threonine.

20 143. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 139 is substituted by alanine.

25 144. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 142 is substituted by threonine.

30 145. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 139 is substituted by threonine.

5 146. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the isoleucine residue at position 102 is substituted by tryptophan.

147. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the alanine residue at position 96 is substituted by asparagine.

5 148. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 42 is substituted by phenylalanine.

10 149. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the serine residue at position 142 is substituted by alanine.

15 150. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the histidine residue at position 118 is substituted by phenylalanine.

20 151. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 237 is substituted by alanine.

25 152. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 255 is substituted by proline.

30 153. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the asparagine residue at position 237 is substituted by alanine and the threonine residue at position 141 is substituted by tryptophan.

35 154. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the threonine residue at position 268 is substituted by valine.

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155. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the lysine residue at position 229 is substituted by tryptophan.

5 156. The hybrid plasmid of claim 105 wherein said mutant gene encodes for said mutant protease wherein the threonine residue at position 141 is substituted by tryptophan.

10 157. A computer based method for identifying the sites which affect the stability of a target protein comprising the steps of: (1) generating a probe-accessible surface of said target protein by probing the three dimensional coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal cavities; (2) identifying the amino acids which make up the boundaries of the internal cavities, wherein said amino acids comprise a set of sites which when mutated increase the stability of the protein.

15 20 158. The computer based method of claim 157 wherein said target protein is *Bacillus lentus* DSM 5483 alkaline protease.

25 30 35 159. A computer based method for identifying the sites which affect the stability of a target protein comprising the steps of: (1) generating a probe-accessible surface of said target protein by probing the three dimensional coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal cavities; (2) aligning said three dimensional coordinates of said target protein and a reference protein by moving the three dimensional coordinates of said reference protein into the coordinate

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frame of said target protein; (3) identifying an amino acid in said reference protein whose side chain lies outside said solvent-accessible surface of said protein or inside said internal cavities of said target protein.

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160. The computer based method of claim 159 wherein said target protein is *Bacillus lentus* DSM 5483 alkaline protease.

10

161. The computer based method of claim 159 wherein said reference protein is any protein for which a three dimensional structure is available which is homologous to the target protein.

15

162. The computer based method of claim 159 wherein said reference protein is thermitase.

163. The computer based method of claim 159 wherein said reference protein is subtilisin Carlsberg.

20

164. The computer based method of claim 159 wherein said reference protein is subtilisin BPN'.

25

165. The computer based method of claim 159 wherein said reference protein is proteinase K.

30

166. A. computer based method for increasing the stability of a protein comprising the steps of: (1) generating a probe-accessible surface of said target protein by probing the coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal cavities; (2) identifying the amino acids which make up the boundaries of the internal cavities, wherein said amino acids comprise a set of sites which when mutated increase the stability of the protein;

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(3) identifying an amino acid mutation which decreases the volume of said internal cavities; (4) determining if said amino acid in said target protein can be changed without creating unacceptable steric interactions; (5) replacing the amino acid in said target protein by site directed mutagenesis of the gene which expresses said target protein.

167. The computer based method of claim 166 wherein said target protein is *Bacillus lentus* DSM 5483 alkaline protease.

168. The computer based method of claim 166 wherein said reference protein is any protein for which a three dimensional structure is available which is homologous to the target protein.

169. The computer based method of claim 166 wherein said reference protein is thermitase.

170. The computer based method of claim 166 wherein said reference protein is subtilisin Carlsberg.

171. The computer based method of claim 166 wherein said reference protein is subtilisin BPN'.

172. The computer based method of claim 166 wherein said reference protein is proteinase K.

173. A computer based method for increasing the stability of a protein comprising the steps of: (1) generating a probe-accessible surface of said target protein by probing the coordinates of said protein with an uncharged probe molecule having a radius of about 0.9 to about 2.0 Å, wherein said probe-accessible surface has an external surface the interior of which contains one or more probe-accessible internal cavities; (2) aligning said three

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dimensional coordinates of said target protein and a reference protein by moving the three dimensional coordinates of said reference protein into the coordinate frame of said target protein; (3) identifying an amino acid in said reference protein whose side chain lies outside said solvent-accessible surface of said protein or inside said internal cavities of said target protein; (4) identifying the amino acid in said target protein which occupies the equivalent position as said amino acid in said reference protein; (5) determining if said amino acid in said target protein can be changed without creating unacceptable steric effects; (6) replacing the amino acid in said target protein with the corresponding amino acid in the equivalent position in said reference protein by site-directed mutagenesis of the gene which expresses said target protein.

174. The computer based method of claim 173 wherein said target protein is *Bacillus lentus* DSM 5483 alkaline protease.

175. The computer based method of claim 173 wherein said reference protein is any protein for which a three dimensional structure is available which is homologous to the target protein.

176. The computer based method of claim 173 wherein said reference protein is thermitase.

177. The computer based method of claim 173 wherein said reference protein is subtilisin Carlsberg.

178. The computer based method of claim 173 wherein said reference protein is subtilisin BPN'.

179. The computer based method of claim 173 wherein said reference protein is proteinase K.

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FIGURE 1

1	GLY	C	27.985	27.065	7.578	8	ILE	O	29.238	35.790	21.181
1	GLY	O	26.834	26.692	7.822	9	SER	N	28.225	36.591	19.284
1	GLY	N	27.785	25.660	5.657	9	SER	CA	29.270	37.572	19.075
1	GLY	CA	28.517	26.825	6.143	9	SER	CB	29.158	38.161	17.652
2	GLN	N	28.745	27.585	8.522	9	SER	CG	29.411	37.107	16.718
2	GLN	CA	28.205	27.868	9.851	9	SER	C	29.191	38.684	20.145
2	GLN	CB	29.179	27.265	10.835	9	SER	O	30.236	39.113	20.660
2	GLN	CG	28.905	27.589	12.287	10	ARG	N	27.977	39.085	20.540
2	GLN	CD	29.834	26.805	13.151	10	ARG	CA	27.775	40.132	21.537
2	GLN	OE1	29.476	25.685	13.540	10	ARG	CB	26.288	40.423	21.686
2	GLN	NE2	31.008	27.317	13.461	10	ARG	CG	25.946	41.656	22.562
2	GLN	C	28.045	29.384	10.049	10	ARG	CD	26.666	42.953	22.101
2	GLN	O	28.927	30.159	9.642	10	ARG	NE	26.378	43.300	20.705
3	SER	N	26.940	29.781	10.693	10	ARG	CZ	25.394	44.138	20.338
3	SER	CA	26.568	31.160	10.999	10	ARG	NH1	25.226	44.365	19.048
3	SER	CB	25.036	31.390	10.712	10	ARG	NH2	24.604	44.767	21.215
3	SER	CG	24.576	30.913	9.455	10	ARG	C	28.351	39.782	22.893
3	SER	C	26.815	31.424	12.488	10	ARG	O	28.942	40.673	23.476
3	SER	O	26.464	30.580	13.314	11	VAL	N	28.222	38.532	23.377
4	VAL	N	27.371	32.570	12.897	11	VAL	CA	28.862	38.186	24.642
4	VAL	CA	27.534	32.913	14.309	11	VAL	CB	28.127	37.003	25.339
4	VAL	CB	28.860	33.625	14.552	11	VAL	CG1	26.664	37.416	25.538
4	VAL	CG1	29.008	33.965	16.045	11	VAL	CG2	28.227	35.723	24.530
4	VAL	CG2	30.006	32.739	14.035	11	VAL	C	30.343	37.832	24.471
4	VAL	C	26.397	33.869	14.655	11	VAL	O	31.021	37.393	25.404
4	VAL	O	26.344	34.990	14.097	12	GLN	N	30.868	37.944	23.261
5	PRO	N	25.384	33.471	15.449	12	GLN	CA	32.288	37.745	22.957
5	PRO	CD	25.140	32.114	15.924	12	GLN	CB	33.129	38.763	23.772
5	PRO	CA	24.313	34.393	15.856	12	GLN	CG	32.773	40.196	23.319
5	PRO	CB	23.404	33.524	16.740	12	GLN	CD	33.643	41.252	23.997
5	PRO	CG	23.629	32.110	16.189	12	GLN	OE1	34.842	41.403	23.753
5	PRO	C	24.823	35.677	16.538	12	GLN	NE2	33.145	42.035	24.926
5	PRO	O	25.816	35.601	17.282	12	GLN	C	32.806	36.330	23.186
6	TRP	N	24.126	36.804	16.302	12	GLN	O	33.978	36.104	23.557
6	TRP	CA	24.597	38.070	16.867	13	ALA	N	31.938	35.350	22.940
6	TRP	CB	23.589	39.231	16.567	13	ALA	CA	32.333	33.978	23.095
6	TRP	CG	22.313	39.360	17.414	13	ALA	CB	31.189	33.004	22.890
6	TRP	CD2	22.238	40.080	18.588	13	ALA	C	33.418	33.589	22.084
6	TRP	CE2	20.905	39.872	18.955	13	ALA	O	34.293	32.789	22.477
6	TRP	CE3	23.091	40.874	19.364	14	PRO	N	33.507	34.053	20.808
6	TRP	CD1	21.120	38.755	17.097	14	PRO	CD	32.522	34.799	20.020
6	TRP	NE1	20.274	39.089	18.047	14	PRO	CA	34.622	33.646	19.943
6	TRP	CZ2	20.485	40.458	20.142	14	PRO	CB	34.311	34.283	18.601
6	TRP	CZ3	22.638	41.455	20.536	14	PRO	CG	32.806	34.270	18.606
6	TRP	CH2	21.339	41.249	20.918	14	PRO	C	35.977	34.034	20.525
6	TRP	C	24.859	38.028	18.378	14	PRO	O	36.900	33.216	20.393
6	TRP	O	25.812	38.610	18.854	15	ALA	N	36.096	35.170	21.257
7	GLY	N	24.056	37.299	19.142	15	ALA	CA	37.383	35.545	21.881
7	GLY	CA	24.171	37.250	20.597	15	ALA	CB	37.253	36.887	22.612
7	GLY	C	25.488	36.591	21.015	15	ALA	C	37.837	34.470	22.892
7	GLY	O	26.135	36.993	22.000	15	ALA	O	39.024	34.129	22.980
8	ILE	N	25.911	35.557	20.242	16	ALA	N	36.899	33.826	23.591
8	ILE	CA	27.125	34.811	20.543	16	ALA	CA	37.248	32.758	24.508
8	ILE	CB	27.250	33.554	19.559	16	ALA	CB	36.057	32.436	25.368
8	ILE	CG2	28.525	32.760	19.882	16	ALA	C	37.632	31.505	23.705
8	ILE	CG1	26.016	32.625	19.654	16	ALA	O	38.587	30.787	24.026
8	ILE	CD	25.683	32.107	21.080	17	HIS	N	36.927	31.180	22.610
8	ILE	C	28.303	35.772	20.363	17	HIS	CA	37.206	29.941	21.872

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FIGURE 1

17	HIS	CB	36.283	29.667	20.715	27	LYS	N	29.799	19.815	32.589
17	HIS	CG	34.810	29.669	21.066	27	LYS	CA	28.459	19.291	32.434
17	HIS	CD2	33.823	29.867	20.140	27	LYS	CB	28.206	18.148	33.370
17	HIS	ND1	34.240	29.557	22.260	27	LYS	CG	29.146	17.001	33.191
17	HIS	CE1	32.932	29.701	22.082	27	LYS	CD	28.427	15.942	33.969
17	HIS	NE2	32.694	29.881	20.807	27	LYS	CE	29.530	15.137	34.529
17	HIS	C	38.577	30.109	21.246	27	LYS	NZ	29.022	14.047	35.345
17	HIS	O	39.290	29.114	21.115	27	LYS	C	27.394	20.331	32.719
18	ASN	N	38.978	31.354	20.903	27	LYS	O	27.368	20.968	33.797
18	ASN	CA	40.320	31.583	20.379	28	VAL	N	26.512	20.472	31.730
18	ASN	CB	40.420	32.976	19.792	28	VAL	CA	25.435	21.471	31.738
18	ASN	CG	39.771	33.007	18.426	28	VAL	CB	25.628	22.534	30.583
18	ASN	OD1	39.324	34.072	17.991	28	VAL	CG1	24.502	23.560	30.598
18	ASN	ND2	39.604	31.952	17.631	28	VAL	CG2	26.989	23.220	30.749
18	ASN	C	41.377	31.382	21.454	28	VAL	C	24.121	20.739	31.512
18	ASN	O	42.545	31.105	21.147	28	VAL	O	23.947	20.067	30.475
19	ARG	N	41.007	31.481	22.726	29	ALA	N	23.203	20.933	32.446
19	ARG	CA	41.934	31.108	23.756	29	ALA	CA	21.900	20.311	32.385
19	ARG	CB	41.579	31.808	25.055	29	ALA	CB	21.478	19.832	33.763
19	ARG	CG	41.755	33.269	24.901	29	ALA	C	20.906	21.382	31.920
19	ARG	CD	41.327	33.963	26.212	29	ALA	O	20.919	22.490	32.454
19	ARG	NE	41.469	35.388	26.008	30	VAL	N	20.038	21.127	30.938
19	ARG	CE	40.620	36.280	26.485	30	VAL	CA	19.069	22.069	30.421
19	ARG	NH1	40.880	37.535	26.211	30	VAL	CB	19.123	22.097	28.835
19	ARG	NH2	39.567	35.963	27.217	30	VAL	CG1	18.017	22.967	28.267
19	ARG	C	41.924	29.600	23.992	30	VAL	CG2	20.480	22.654	28.369
19	ARG	O	42.655	29.144	24.864	30	VAL	C	17.731	21.519	30.928
20	GLY	N	41.166	28.766	23.312	30	VAL	O	17.275	20.467	30.425
20	GLY	CA	41.105	27.344	23.620	31	LEU	N	17.155	22.192	31.928
20	GLY	C	40.056	26.959	24.682	31	LEU	CA	15.899	21.751	32.514
20	GLY	O	40.026	25.824	25.187	31	LEU	CB	15.878	22.118	33.997
21	LEU	N	39.130	27.872	25.003	31	LEU	CG	16.523	21.135	34.997
21	LEU	CA	38.098	27.626	26.023	31	LEU	CD1	18.034	21.230	34.828
21	LEU	CB	38.012	28.796	26.984	31	LEU	CD2	16.177	21.487	36.457
21	LEU	CG	39.321	29.049	27.732	31	LEU	C	14.832	22.501	31.724
21	LEU	CD1	39.370	30.463	28.219	31	LEU	O	14.647	23.705	31.887
21	LEU	CD2	39.469	28.017	28.815	32	ASP	N	14.163	21.816	30.801
21	LEU	C	36.767	27.463	25.284	32	ASP	CA	13.254	22.474	29.860
21	LEU	O	36.254	28.371	24.622	32	ASP	CB	14.173	23.197	28.850
22	THR	N	36.294	26.227	25.368	32	ASP	CG	13.567	24.470	28.221
22	THR	CA	35.094	25.767	24.713	32	ASP	OD1	14.128	25.565	28.394
22	THR	CB	35.488	24.785	23.658	32	ASP	OD2	12.549	24.352	27.538
22	THR	OG1	36.139	23.695	24.331	32	ASP	C	12.331	21.405	29.226
22	THR	OG2	36.341	25.467	22.585	32	ASP	O	12.057	20.382	29.870
22	THR	C	34.069	25.126	25.622	33	THR	N	11.874	21.602	27.972
22	THR	O	33.010	24.745	25.146	33	THR	CA	10.956	20.709	27.245
23	GLY	N	34.304	24.953	26.918	33	THR	CB	10.237	21.562	26.131
23	GLY	CA	33.327	24.232	27.761	33	THR	OG1	11.275	22.099	25.255
23	GLY	C	33.680	22.769	27.973	33	THR	OG2	9.394	22.669	26.737
23	GLY	O	32.931	22.033	28.642	33	THR	C	11.600	19.465	26.594
24	SER	N	34.808	22.329	27.403	33	THR	O	10.948	18.766	25.806
24	SER	CA	35.218	20.939	27.546	34	GLY	N	12.919	19.306	26.830
24	SER	CB	36.565	20.776	26.874	34	GLY	CA	13.720	18.216	26.294
24	SER	OG	36.819	19.378	26.828	34	GLY	C	14.758	18.794	25.334
24	SER	C	35.310	20.485	29.016	34	GLY	O	14.875	20.030	25.242
24	SER	O	35.830	21.218	29.880	35	ILE	N	15.492	17.921	24.630
25	GLY	N	34.786	19.290	29.245	35	ILE	CA	16.417	18.299	23.557
25	GLY	CA	34.688	18.702	30.571	35	ILE	CB	17.881	18.366	24.013
25	GLY	C	33.657	19.387	31.517	35	ILE	CG2	18.614	19.017	22.822
25	GLY	O	33.562	19.018	32.697	35	ILE	CG1	18.149	19.249	25.273
26	VAL	N	32.861	20.356	31.079	35	ILE	CD	19.589	19.096	25.859
26	VAL	CA	31.862	20.949	31.956	35	ILE	C	16.257	17.256	22.439
26	VAL	CB	31.863	22.501	31.794	35	ILE	O	16.348	16.042	22.687
26	VAL	CG1	30.812	23.111	32.729	36	SER	N	15.873	17.729	21.243
26	VAL	CG2	32.281	23.055	32.071	36	SER	CA	15.797	16.820	20.059
26	VAL	C	30.488	20.382	31.604	36	SER	CB	14.885	17.400	19.036
26	VAL	O	30.089	20.375	30.446	36	SER	CG	13.589	17.293	19.580

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36	SEK	C	17.166	16.572	19.462	44	ARG	C	24.399	12.088	27.123
36	SEK	O	18.018	17.473	19.331	44	ARG	O	24.863	11.030	27.534
37	THR	N	17.380	15.298	19.076	45	GLY	N	23.168	12.489	27.392
37	THR	CA	18.541	14.930	18.274	45	GLY	CA	22.286	11.766	28.306
37	THR	CB	18.300	13.522	17.755	45	GLY	C	21.220	12.697	28.867
37	THR	CG1	18.169	12.722	18.926	45	GLY	O	21.009	13.824	28.377
37	THR	CG2	19.401	13.039	16.808	46	GLY	N	20.524	12.208	29.871
37	THR	C	18.675	15.912	17.089	46	GLY	CA	19.453	12.976	30.489
37	THR	O	17.670	16.153	16.374	46	GLY	C	18.430	12.113	31.221
38	HIS	N	19.880	16.435	16.837	46	GLY	O	18.632	10.912	31.445
38	HIS	CA	20.021	17.474	15.806	47	ALA	N	17.313	12.744	31.558
38	HIS	CB	19.786	18.868	16.461	47	ALA	CA	16.222	12.120	32.291
38	HIS	CG	19.722	20.046	15.486	47	ALA	CB	16.461	12.192	33.779
38	HIS	CG2	20.803	20.545	14.801	47	ALA	C	14.953	12.896	31.997
38	HIS	ND1	18.655	20.775	15.114	47	ALA	O	15.007	14.081	31.604
38	HIS	CE1	19.051	21.670	14.239	48	SEK	N	13.817	12.215	32.075
38	HIS	NE2	20.348	21.530	14.048	48	SEK	CA	12.537	12.888	31.947
38	HIS	C	21.432	17.344	15.305	48	SEK	CB	11.680	12.343	30.801
38	HIS	O	22.341	17.174	16.118	48	SEK	CG	10.390	12.945	30.842
39	PRO	N	21.740	17.555	14.025	48	SEK	C	11.760	12.680	33.243
39	PRO	CD	20.795	17.752	12.918	48	SEK	O	11.740	11.558	33.791
39	PRO	CA	23.135	17.467	13.571	49	PHE	N	11.224	13.808	33.696
39	PRO	CB	23.084	17.619	12.070	49	PHE	CA	10.358	13.821	34.885
39	PRO	CG	21.744	18.261	11.799	49	PHE	CB	10.967	14.782	35.924
39	PRO	C	24.112	18.457	14.195	49	PHE	CG	12.302	14.253	36.403
39	PRO	O	25.318	18.260	14.162	49	PHE	CD1	13.454	14.844	35.923
40	ASP	N	23.645	19.520	14.832	49	PHE	CD2	12.383	13.128	37.204
40	ASP	CA	24.583	20.488	15.375	49	PHE	CE1	14.676	14.300	36.225
40	ASP	CB	24.218	21.897	14.900	49	PHE	CE2	13.616	12.590	37.509
40	ASP	CG	25.453	22.801	14.740	49	PHE	CZ	14.760	13.176	37.008
40	ASP	OD1	26.526	22.264	14.551	49	PHE	C	8.915	14.206	34.546
40	ASP	OD2	25.389	24.037	14.740	49	PHE	O	8.115	14.601	35.418
40	ASP	C	24.561	20.439	16.874	50	VAL	N	8.571	14.104	33.248
40	ASP	O	24.918	21.450	17.480	50	VAL	CA	7.230	14.424	32.796
41	LEU	N	24.080	19.327	17.430	50	VAL	CB	7.264	15.245	31.450
41	LEU	CA	24.102	19.142	18.883	50	VAL	CG1	5.869	15.427	30.821
41	LEU	CB	22.713	19.260	19.513	50	VAL	CG2	7.766	16.635	31.755
41	LEU	CG	21.938	20.541	19.465	50	VAL	C	6.512	13.085	32.594
41	LEU	CD1	20.485	20.249	19.882	50	VAL	O	6.894	12.336	31.695
41	LEU	CD2	22.642	21.595	20.331	51	PRO	N	5.443	12.724	33.315
41	LEU	C	24.635	17.780	19.265	51	PRO	CD	4.826	13.553	34.344
41	LEU	O	24.417	16.802	18.530	51	PRO	CA	4.805	11.411	33.232
42	ASN	N	25.298	17.707	20.415	51	PRO	CB	3.632	11.476	34.218
42	ASN	CA	25.792	16.443	20.953	51	PRO	CG	4.118	12.525	35.235
42	ASN	CB	27.341	16.452	21.066	51	PRO	C	4.358	10.971	31.854
42	ASN	CG	27.960	15.195	21.667	51	PRO	O	4.621	9.848	31.454
42	ASN	OD1	29.168	15.169	21.967	52	GLY	N	3.693	11.820	31.082
42	ASN	ND2	27.260	14.090	21.803	52	GLY	CA	3.269	11.377	29.746
42	ASN	C	25.176	16.272	22.354	52	GLY	C	4.368	11.323	28.690
42	ASN	O	25.590	16.890	23.332	52	GLY	O	4.117	10.848	27.575
43	ILE	N	24.152	15.442	22.457	53	GLU	N	5.606	11.757	28.996
43	ILE	CA	23.458	15.252	23.736	53	GLU	CA	6.645	11.848	28.005
43	ILE	CB	21.958	15.077	23.423	53	GLU	CB	6.909	13.311	27.676
43	ILE	CG2	21.208	14.865	24.766	53	GLU	CG	5.740	13.985	27.008
43	ILE	CG1	21.451	16.284	22.605	53	GLU	CD	5.991	15.433	26.597
43	ILE	CD	20.150	16.044	21.857	53	GLU	OE1	7.145	15.826	26.393
43	ILE	C	24.075	14.023	24.422	53	GLU	OE2	5.012	16.167	26.462
43	ILE	O	24.160	12.963	23.781	53	GLU	C	7.901	11.202	28.519
44	ARG	N	24.520	14.131	25.675	54	PRO	N	8.803	11.919	28.919
44	ARG	CA	25.246	13.030	26.309	54	PRO	CD	8.059	9.880	28.483
44	ARG	CB	26.332	13.557	27.250	54	PRO	CA	7.103	8.945	27.908
44	ARG	CG	27.060	14.753	26.730	54	PRO	CB	9.245	9.200	29.004
44	ARG	CD	27.731	14.330	25.467	54	PRO	CG	8.817	7.745	28.993
44	ARG	NE	29.007	13.812	25.844	54	PRO	C	7.964	7.702	27.752
44	ARG	CZ	30.106	14.554	25.653	54	PRO	O	10.548	9.487	28.240
44	ARG	NE1	31.274	14.034	26.023	54	PRO	N	11.625	9.174	28.750
44	ARG	NE2	30.099	15.758	25.065	55	SEK	N	10.497	10.048	27.015

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55	SKR	CA	11.678	10.360	26.197	65	HIS	CA	16.749	26.168	20.989
55	SKR	CB	11.310	10.444	24.730	65	HIS	CB	15.534	27.012	20.769
55	SKR	CG	12.390	10.759	23.870	65	HIS	CG	15.850	28.409	20.237
55	SKR	C	12.250	11.702	26.559	65	HIS	CD2	15.686	28.794	18.918
55	SKR	O	11.469	12.540	27.001	65	HIS	ND1	16.319	29.457	20.941
56	THR	N	13.533	11.968	26.265	65	HIS	CE1	16.438	30.455	20.096
56	THR	CA	14.084	13.315	26.487	65	HIS	NE2	16.056	30.048	18.887
56	THR	CB	15.596	13.250	26.945	65	HIS	C	17.672	26.657	22.118
56	THR	CG1	16.283	12.433	25.998	65	HIS	O	18.820	27.073	21.904
56	THR	CG2	15.743	12.741	28.390	66	VAL	N	17.220	26.535	23.376
56	THR	C	13.978	14.192	25.225	66	VAL	CA	18.084	26.803	24.544
56	THR	O	14.370	15.358	25.250	66	VAL	CB	17.351	26.378	25.832
57	GLN	N	13.331	13.623	24.170	66	VAL	CG1	18.194	26.482	27.092
57	GLN	CA	13.252	14.317	22.886	66	VAL	CG2	16.264	27.335	25.994
57	GLN	CB	12.743	13.375	21.797	66	VAL	C	19.427	26.062	24.466
57	GLN	CG	13.825	12.370	21.360	66	VAL	O	20.494	26.687	24.586
57	GLN	CD	15.108	13.013	20.762	67	ALA	N	19.347	24.730	24.292
57	GLN	OE1	15.091	13.752	19.766	67	ALA	CA	20.534	23.878	24.204
57	GLN	NE2	16.267	12.793	21.390	67	ALA	CB	20.081	22.462	23.828
57	GLN	C	12.314	15.495	23.027	67	ALA	C	21.526	24.393	23.140
57	GLN	O	11.395	15.425	23.858	67	ALA	O	22.732	24.464	23.385
58	ASP	N	12.508	16.545	22.256	68	GLY	N	21.028	24.843	21.978
58	ASP	CA	11.724	17.738	22.451	68	GLY	CA	21.890	25.373	20.923
58	ASP	CB	12.619	18.910	22.214	68	GLY	C	22.602	26.682	21.221
58	ASP	CG	12.036	20.302	22.427	68	GLY	O	23.730	26.888	20.726
58	ASP	OD1	10.950	20.447	23.006	69	THR	N	22.009	27.580	22.020
58	ASP	OD2	12.737	21.245	22.032	69	THR	CA	22.727	28.785	22.414
58	ASP	C	10.499	17.854	21.573	69	THR	CB	21.703	29.733	23.084
58	ASP	O	10.627	18.076	20.358	69	THR	CG1	20.690	29.972	22.076
59	GLY	N	9.311	17.809	22.191	69	THR	CG2	22.339	31.046	23.576
59	GLY	CA	8.021	17.992	21.500	69	THR	C	23.902	28.431	23.353
59	GLY	C	7.601	19.445	21.318	69	THR	O	24.986	29.042	23.288
59	GLY	O	6.527	19.731	20.754	70	ILE	N	23.686	27.426	24.235
60	ASN	N	8.431	20.374	21.802	70	ILE	CA	24.771	26.952	25.107
60	ASN	CA	8.085	21.787	21.793	70	ILE	CB	24.305	25.947	26.219
60	ASN	CB	8.166	22.340	23.222	70	ILE	CG2	25.501	25.525	27.092
60	ASN	CG	7.768	23.804	23.268	70	ILE	CG1	23.197	26.607	27.065
60	ASN	OD1	8.585	24.702	23.090	70	ILE	CD	22.458	25.687	28.103
60	ASN	ND2	6.503	24.085	23.545	70	ILE	C	25.820	26.222	24.285
60	ASN	C	8.971	22.642	20.883	70	ILE	O	27.014	26.530	24.398
60	ASN	O	8.525	23.378	20.022	71	ALA	N	25.447	25.251	23.451
61	GLY	N	10.269	22.585	21.093	71	ALA	CA	26.467	24.349	22.986
61	GLY	CA	11.202	23.372	20.337	71	ALA	CB	26.523	23.129	23.948
61	GLY	C	12.035	24.187	21.318	71	ALA	C	26.352	23.895	21.578
61	GLY	O	13.231	24.429	21.115	71	ALA	O	26.869	22.805	21.295
62	HIS	N	11.417	24.583	22.439	72	ALA	N	25.785	24.709	20.671
62	HIS	CA	12.068	25.515	23.336	72	ALA	CA	25.772	24.252	19.280
62	HIS	CB	11.034	25.886	24.385	72	ALA	CB	25.105	25.252	18.367
62	HIS	CG	11.450	27.020	25.268	72	ALA	C	27.223	24.056	18.832
62	HIS	CD2	11.218	28.363	25.048	72	ALA	O	28.112	24.803	19.205
62	HIS	ND1	11.969	26.858	26.498	73	LEU	N	27.412	22.934	18.090
62	HIS	CE1	12.011	28.039	27.067	73	LEU	CA	28.744	22.458	17.726
62	HIS	NE2	11.572	28.932	26.189	73	LEU	CB	28.630	21.030	17.087
62	HIS	C	13.371	24.957	23.944	73	LEU	CG	27.913	19.969	17.918
62	HIS	O	14.409	25.642	23.918	73	LEU	CD1	27.805	18.638	17.193
63	GLY	N	13.351	23.723	24.453	73	LEU	CD2	28.650	19.898	19.221
63	GLY	CA	14.577	23.186	25.039	73	LEU	C	29.465	23.384	16.782
63	GLY	C	15.709	23.028	24.021	73	LEU	O	28.857	23.968	15.858
63	GLY	O	16.870	23.232	24.356	74	ASN	N	30.768	23.410	17.002
64	THR	N	15.375	22.712	22.746	74	ASN	CA	31.650	24.268	16.196
64	THR	CA	16.392	22.485	21.700	74	ASN	CB	32.829	24.736	17.002
64	THR	CB	15.729	21.894	20.395	74	ASN	CG	33.638	25.786	16.240
64	THR	CG1	15.057	20.682	20.709	74	ASN	OD1	33.278	26.358	15.207
64	THR	CG2	16.823	21.570	19.338	74	ASN	ND2	34.798	26.098	16.774
64	THR	C	17.078	23.790	21.373	74	ASN	C	32.170	23.435	15.022
64	THR	O	18.287	23.810	21.100	74	ASN	O	33.097	22.639	15.197
65	HIS	N	16.252	24.838	21.308	75	ASN	N	31.602	23.663	13.836

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FIGURE 1

75	ASN	CA	31.833	22.805	12.665	85	SER	C	31.718	21.142	25.147
75	ASN	CB	30.957	21.533	12.702	85	SER	O	32.117	20.128	25.689
75	ASN	CG	29.491	21.798	13.007	86	ALA	N	30.806	21.904	25.738
75	ASN	OD1	28.914	22.891	12.815	86	ALA	CA	30.151	21.459	26.967
75	ASN	ND2	28.869	20.780	13.605	86	ALA	CB	29.153	22.489	27.391
75	ASN	C	31.502	23.581	11.415	86	ALA	C	29.408	20.122	26.787
75	ASN	O	31.582	24.793	11.490	86	ALA	O	28.914	19.771	25.689
76	SER	N	31.121	22.947	10.298	87	GLU	N	29.338	19.367	27.882
76	SER	CA	30.794	23.635	9.055	87	GLU	CA	28.637	18.102	27.917
76	SER	CB	31.452	22.852	7.920	87	GLU	CB	29.274	17.235	28.985
76	SER	CG	32.867	22.956	8.023	87	GLU	CG	30.727	16.977	28.652
76	SER	C	29.308	23.826	8.771	87	GLU	CD	31.359	15.911	29.523
76	SER	O	28.913	24.172	7.628	87	GLU	OE1	30.638	15.142	30.165
77	ILE	N	28.486	23.612	9.815	87	GLU	OE2	32.580	15.850	29.550
77	ILE	CA	27.049	23.710	9.658	87	GLU	C	27.172	18.407	28.237
77	ILE	CB	26.315	22.283	9.597	87	GLU	O	26.787	18.788	29.353
77	ILE	CG2	26.735	21.594	8.269	88	LEU	N	26.340	18.241	27.230
77	ILE	CG1	26.604	21.393	10.803	88	LEU	CA	24.949	18.654	27.326
77	ILE	CD	25.657	20.178	10.887	88	LEU	CB	24.566	19.080	25.910
77	ILE	C	26.407	24.494	10.799	88	LEU	CG	23.561	20.137	25.626
77	ILE	O	26.960	24.700	11.891	88	LEU	CD1	23.929	21.475	26.321
78	GLY	N	25.199	24.925	10.501	88	LEU	CD2	23.521	20.293	24.093
78	GLY	CA	24.338	25.534	11.486	88	LEU	C	24.042	17.570	27.876
78	GLY	C	24.874	26.773	12.159	88	LEU	O	24.093	16.491	27.282
78	GLY	O	25.345	27.713	11.542	89	TYR	N	23.223	17.777	28.919
79	VAL	N	24.781	26.721	13.475	89	TYR	CA	22.249	16.807	29.449
79	VAL	CA	25.226	27.840	14.293	89	TYR	CB	22.538	16.474	30.942
79	VAL	CB	23.977	28.470	15.058	89	TYR	CG	23.828	15.673	31.047
79	VAL	CG1	23.105	29.130	14.034	89	TYR	CD1	25.048	16.317	30.920
79	VAL	CG2	23.172	27.468	15.841	89	TYR	CE1	26.230	15.627	30.860
79	VAL	C	26.342	27.460	15.258	89	TYR	CD2	23.797	14.292	31.142
79	VAL	O	27.035	26.445	15.015	89	TYR	CE2	24.979	13.578	31.070
80	LEU	N	26.574	28.266	16.310	89	TYR	CZ	26.175	14.250	30.937
80	LEU	CA	27.681	28.023	17.216	89	TYR	OH	27.340	13.513	30.872
80	LEU	CB	28.856	28.882	16.777	89	TYR	C	20.847	17.347	29.318
80	LEU	CG	30.090	28.886	17.612	89	TYR	O	20.561	18.513	29.646
80	LEU	CD1	30.630	27.510	17.592	90	ALA	N	20.000	16.511	28.733
80	LEU	CD2	31.076	29.900	17.113	90	ALA	CA	18.613	16.880	28.538
80	LEU	C	27.210	28.436	18.614	90	ALA	CB	17.991	16.206	27.306
80	LEU	O	26.667	29.536	18.725	90	ALA	C	17.794	16.453	29.749
81	GLY	N	27.333	27.597	19.625	90	ALA	O	17.565	15.260	29.984
81	GLY	CA	26.928	28.085	20.924	91	VAL	N	17.307	17.405	30.542
81	GLY	C	28.076	28.805	21.662	91	VAL	CA	16.489	17.070	31.706
81	GLY	O	29.253	28.863	21.248	91	VAL	CB	17.050	17.737	32.979
82	VAL	N	27.794	29.222	22.883	91	VAL	CG1	16.278	17.172	34.186
82	VAL	CA	28.824	29.876	23.663	91	VAL	CG2	18.529	17.434	33.152
82	VAL	CB	28.207	30.550	24.929	91	VAL	C	15.086	17.576	31.413
82	VAL	CG1	29.266	31.108	25.913	91	VAL	O	14.803	18.789	31.545
82	VAL	CG2	27.250	31.691	24.395	92	LYS	N	14.186	16.716	30.935
82	VAL	C	29.915	28.926	24.085	92	LYS	CA	12.860	17.211	30.608
82	VAL	O	31.102	29.295	24.118	92	LYS	CB	12.271	16.257	29.604
83	ALA	N	29.504	27.716	24.494	92	LYS	CG	10.802	16.621	29.273
83	ALA	CA	30.437	26.706	24.970	92	LYS	CD	10.070	15.579	28.398
83	ALA	CB	30.194	26.444	26.456	92	LYS	CE	10.580	15.652	26.970
83	ALA	C	30.270	25.404	24.181	92	LYS	NZ	9.873	14.730	26.095
83	ALA	O	29.605	24.459	24.615	92	LYS	C	12.009	17.347	31.892
84	PRO	N	30.827	25.356	22.956	92	LYS	O	11.719	16.396	32.624
84	PRO	CD	31.627	26.423	22.334	93	VAL	N	11.659	18.596	32.162
84	PRO	CA	30.449	24.325	21.985	93	VAL	CA	10.834	18.966	33.299
84	PRO	CB	30.988	24.826	20.658	93	VAL	CB	11.520	19.956	34.315
84	PRO	CG	31.954	25.925	20.928	93	VAL	CG1	12.719	19.267	34.948
84	PRO	C	30.900	22.929	22.328	93	VAL	CG2	11.808	21.301	33.634
84	PRO	O	30.460	21.987	21.673	93	VAL	C	9.545	19.632	32.844
85	SER	N	31.795	22.800	23.311	93	VAL	O	8.636	19.907	33.627
85	SER	CA	32.303	21.525	23.810	94	LEU	N	9.434	19.988	31.564
85	SER	CB	33.826	21.574	23.944	94	LEU	CA	8.253	20.623	31.522
85	SER	CG	34.358	21.691	22.630	94	LEU	CB	8.576	22.025	30.524

FIGURE 1

94 LEU	CG	9.291	22.983	31.432	105 ILE	CA	11.308	20.992	38.055
94 LEU	CD1	9.772	24.188	30.604	105 ILE	CB	10.782	22.425	38.033
94 LEU	CD2	8.380	23.374	32.555	105 ILE	CG2	12.002	23.365	38.118
94 LEU	C	7.783	19.781	29.830	105 ILE	CG1	9.919	22.652	36.794
95 GLY	N	8.605	19.154	29.150	105 ILE	CD	9.191	24.036	36.796
95 GLY	CA	6.479	19.754	29.581	105 ILE	C	12.186	20.703	39.293
95 GLY	C	5.913	18.985	28.494	105 ILE	O	13.406	20.539	39.166
95 GLY	O	5.987	19.713	27.150	106 ALA	N	11.585	20.494	40.484
96 ALA	N	6.394	20.881	27.052	106 ALA	CA	12.324	20.165	41.677
96 ALA	CA	5.518	18.995	26.112	106 ALA	CB	11.347	20.164	42.870
96 ALA	CB	5.460	19.485	24.733	106 ALA	C	13.009	18.797	41.505
96 ALA	C	4.826	18.408	23.824	106 ALA	O	14.185	18.706	41.872
96 ALA	O	4.659	20.791	24.611	107 GLN	N	12.452	17.715	40.904
97 ASP	N	4.945	21.657	23.772	107 GLN	CA	13.267	16.487	40.797
97 ASP	CA	3.680	20.986	25.508	107 GLN	CB	12.484	15.170	40.501
97 ASP	CB	2.957	22.248	25.636	107 GLN	CG	11.380	14.761	41.453
97 ASP	CG	1.637	22.010	26.330	107 GLN	CD	10.582	13.516	41.085
97 ASP	OD1	1.665	21.267	27.665	107 GLN	OE1	9.435	13.412	41.526
97 ASP	OD2	2.704	20.782	28.130	107 GLN	NE2	11.040	12.542	40.292
97 ASP	C	0.596	21.183	28.270	107 GLN	C	14.299	16.625	39.702
97 ASP	O	3.645	23.410	26.351	107 GLN	O	15.333	15.973	39.804
97 ASP	N	3.058	24.477	26.509	108 GLY	N	14.058	17.494	38.722
98 GLY	N	4.885	23.232	26.820	108 GLY	CA	15.068	17.832	37.732
98 GLY	CA	5.597	24.264	27.561	108 GLY	C	16.281	18.376	38.456
98 GLY	C	5.223	24.311	29.038	108 GLY	O	17.409	17.922	38.169
98 GLY	O	5.866	24.997	29.828	109 LEU	N	16.086	19.337	39.380
99 ARG	N	4.228	23.548	29.442	109 LEU	CA	17.203	19.921	40.151
99 ARG	CA	3.746	23.492	30.813	109 LEU	CB	16.703	21.098	40.941
99 ARG	CB	2.274	23.049	30.885	109 LEU	CG	16.358	22.306	40.103
99 ARG	CG	1.275	23.728	29.965	109 LEU	CD1	15.553	23.267	40.958
99 ARG	CD	1.373	25.198	30.169	109 LEU	CD2	17.613	22.976	39.579
99 ARG	NE	0.065	25.771	29.978	109 LEU	C	17.899	18.952	41.088
99 ARG	CZ	-0.085	27.070	29.703	109 LEU	O	19.137	18.923	41.163
99 ARG	NH1	-1.339	27.516	29.555	110 GLU	N	17.146	18.078	41.739
99 ARG	NH2	0.956	27.923	29.560	110 GLU	CA	17.767	16.997	42.502
99 ARG	C	4.518	22.498	31.672	110 GLU	CB	16.706	16.208	43.295
99 ARG	O	4.851	21.418	31.175	110 GLU	CG	16.044	17.043	44.443
100 GLY	N	4.746	22.767	32.962	110 GLU	CD	16.869	17.518	45.693
100 GLY	CA	5.370	21.790	33.846	110 GLU	OE1	16.284	18.250	46.507
100 GLY	C	5.043	22.002	35.327	110 GLU	OE2	18.058	17.205	45.884
100 GLY	O	4.933	23.136	35.803	110 GLU	C	18.562	16.049	41.616
101 ALA	N	4.881	20.881	36.029	110 GLU	O	19.674	15.702	42.025
101 ALA	CA	4.592	20.897	37.462	111 TRP	N	18.111	15.691	40.389
101 ALA	CB	4.090	19.544	37.966	111 TRP	CA	18.867	14.850	39.469
101 ALA	C	5.844	21.210	38.278	111 TRP	CB	18.049	14.586	38.169
101 ALA	O	6.945	20.745	37.930	111 TRP	CG	18.743	13.709	37.091
102 ILE	N	5.672	21.920	39.412	111 TRP	CD2	19.617	14.121	36.111
102 ILE	CA	6.812	22.262	40.268	111 TRP	CE2	19.919	12.914	35.467
102 ILE	CB	6.297	23.134	41.461	111 TRP	CE3	20.195	15.302	35.658
102 ILE	CG2	7.414	23.536	42.429	111 TRP	CD1	18.535	12.343	37.029
102 ILE	CG1	5.672	24.383	40.856	111 TRP	NE1	19.264	11.895	36.042
102 ILE	CD	6.675	25.257	40.045	111 TRP	CZ2	20.803	12.903	34.389
102 ILE	C	7.555	21.016	40.763	111 TRP	CZ3	21.073	15.292	34.585
102 ILE	O	8.790	21.014	40.848	111 TRP	CH2	21.370	14.099	33.959
103 SER	N	6.839	19.922	41.067	111 TRP	C	20.160	15.563	39.124
103 SER	CA	7.477	18.691	41.459	111 TRP	O	21.198	14.910	39.072
103 SER	CB	6.399	17.659	41.711	112 ALA	N	20.134	16.881	38.876
103 SER	OG	5.570	17.479	40.562	112 ALA	CA	21.331	17.620	38.528
103 SER	C	8.451	18.211	40.361	112 ALA	CB	21.029	19.102	38.310
103 SER	O	9.575	17.820	40.676	112 ALA	C	22.411	17.530	39.612
104 SER	N	8.068	18.299	39.085	112 ALA	O	23.578	17.183	39.356
104 SER	CA	8.950	17.948	37.972	113 GLY	N	22.019	17.742	40.859
104 SER	CB	8.185	18.077	36.660	113 GLY	CA	22.962	17.686	41.947
104 SER	OG	7.214	17.048	36.535	113 GLY	C	23.404	16.258	42.205
104 SER	C	10.230	18.802	37.897	113 GLY	O	24.567	16.052	42.565
104 SER	O	11.330	18.272	37.756	114 ASN	N	22.524	15.285	42.009
105 ILE	N	10.136	20.124	38.041	114 ASN	CA	22.901	13.872	42.121

SUBSTITUTE SHEET

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FIGURE 1

114 ASN	CB	21.735	12.858	42.176	123 SER	OG	16.514	29.408	29.479
114 ASN	CG	20.764	12.994	43.318	123 SER	C	13.240	29.029	31.383
114 ASN	OD1	21.095	13.531	44.373	123 SER	O	12.521	28.751	30.400
114 ASN	ND2	19.511	12.575	43.163	124 LEU	N	12.818	29.236	32.647
114 ASN	C	23.820	13.339	41.111	124 LEU	CA	11.426	29.119	33.059
114 ASN	O	24.532	12.346	41.311	124 LEU	CB	11.093	27.646	33.233
115 ASN	N	23.767	13.953	39.923	124 LEU	CG	12.008	26.810	34.115
115 ASN	CA	24.558	13.494	38.817	124 LEU	CD1	11.540	26.904	35.610
115 ASN	CB	23.678	13.382	37.576	124 LEU	CD2	11.993	25.356	33.606
115 ASN	CG	22.871	12.090	37.637	124 LEU	C	11.200	29.897	34.347
115 ASN	OD1	23.296	11.044	37.144	124 LEU	O	12.165	30.261	35.045
115 ASN	ND2	21.716	12.088	38.291	125 GLY	N	9.951	30.177	34.709
115 ASN	C	25.761	14.354	38.510	125 GLY	CA	9.733	31.019	35.884
115 ASN	O	26.352	14.277	37.428	125 GLY	C	8.243	31.204	36.140
116 GLY	N	26.126	15.225	39.431	125 GLY	O	7.396	31.003	35.252
116 GLY	CA	27.354	15.971	39.331	126 SER	N	7.991	31.643	37.370
116 GLY	C	27.372	16.991	38.204	126 SER	CA	6.640	31.772	37.888
116 GLY	O	28.450	17.247	37.614	126 SER	CB	6.331	30.503	38.752
117 MET	N	26.235	17.614	37.909	126 SER	OG	5.242	30.673	39.682
117 MET	CA	26.210	18.667	36.878	126 SER	C	6.623	33.055	38.707
117 MET	CB	24.807	19.105	36.509	126 SER	O	7.650	33.353	39.302
117 MET	CG	23.929	18.029	35.895	127 PRO	N	5.544	33.844	38.839
117 MET	SD	24.529	17.426	34.290	127 PRO	CD	4.300	33.663	38.088
117 MET	CE	24.874	15.741	34.705	127 PRO	CA	5.458	35.005	39.740
117 MET	C	26.888	19.893	37.466	127 PRO	CB	4.310	35.813	39.157
117 MET	O	26.805	20.170	38.688	127 PRO	CG	3.377	34.706	38.715
118 HIS	N	27.549	20.672	36.615	127 PRO	C	5.258	34.663	41.234
118 HIS	CA	28.186	21.879	37.094	127 PRO	O	5.342	35.518	42.119
118 HIS	CB	29.481	22.174	36.318	128 SER	N	4.904	33.408	41.511
118 HIS	CG	30.504	21.026	36.418	128 SER	CA	4.673	32.939	42.860
118 HIS	CD2	30.795	20.176	35.397	128 SER	CB	3.340	32.142	42.821
118 HIS	ND1	31.283	20.653	37.437	128 SER	OG	2.292	33.013	42.389
118 HIS	CE1	32.020	19.622	37.044	128 SER	C	5.845	32.100	43.399
118 HIS	NE2	31.715	19.339	35.797	128 SER	O	6.430	31.293	42.646
118 HIS	C	27.256	23.067	36.967	129 PRO	N	6.223	32.275	44.678
118 HIS	O	27.293	23.989	37.781	129 PRO	CD	5.713	33.322	45.595
119 VAL	N	26.349	23.070	35.989	129 PRO	CA	7.185	31.419	45.363
119 VAL	CA	25.540	24.246	35.723	129 PRO	CB	7.492	32.187	46.641
119 VAL	CB	26.124	25.082	34.533	129 PRO	CG	6.138	32.757	46.937
119 VAL	CG1	25.194	26.267	34.244	129 PRO	C	6.639	29.999	45.605
119 VAL	CG2	27.537	25.612	34.864	129 PRO	O	5.416	29.779	45.693
119 VAL	C	24.194	23.670	35.344	130 SER	N	7.567	29.069	45.789
119 VAL	O	24.123	22.627	34.674	130 SER	CA	7.242	27.724	46.139
120 ALA	N	23.150	24.305	35.817	130 SER	CB	7.197	26.894	44.888
120 ALA	CA	21.801	23.917	35.457	130 SER	OG	7.387	25.528	45.215
120 ALA	CB	21.074	23.434	36.689	130 SER	C	8.260	27.146	47.092
120 ALA	C	21.128	25.170	34.893	130 SER	O	9.462	27.127	46.751
120 ALA	O	21.156	26.255	35.503	131 ALA	N	7.759	26.596	48.220
121 ASN	N	20.621	25.061	33.673	131 ALA	CA	8.619	25.896	49.154
121 ASN	CA	19.917	26.133	32.994	131 ALA	CB	7.818	25.334	50.312
121 ASN	CB	20.330	26.144	31.516	131 ALA	C	9.445	24.755	48.557
121 ASN	CG	19.771	27.348	30.778	131 ALA	O	10.670	24.654	48.755
121 ASN	OD1	20.464	28.304	30.514	132 THR	N	8.761	23.973	47.716
121 ASN	ND2	18.511	27.315	30.418	132 THR	CA	9.373	22.810	47.044
121 ASN	C	18.399	25.942	33.133	132 THR	CB	8.274	22.155	46.232
121 ASN	O	17.793	24.936	32.715	132 THR	OG1	7.351	21.804	47.256
122 LEU	N	17.740	26.917	33.768	132 THR	CG2	8.667	20.937	45.371
122 LEU	CA	16.277	26.942	33.962	132 THR	C	10.547	23.223	46.156
122 LEU	CB	15.895	27.041	35.454	132 THR	O	11.674	22.711	46.213
122 LEU	CG	16.010	25.856	36.340	133 LEU	N	10.257	24.266	45.394
122 LEU	CD1	15.879	26.350	37.770	133 LEU	CA	11.185	24.742	44.396
122 LEU	CD2	14.914	24.875	36.068	133 LEU	CB	10.467	25.753	43.511
122 LEU	C	15.706	28.182	33.264	133 LEU	CG	11.231	26.287	42.326
122 LEU	O	15.618	29.298	33.808	133 LEU	CD1	11.504	25.174	41.324
123 SER	N	15.297	28.013	32.012	133 LEU	CD2	10.395	27.377	41.667
123 SER	CA	14.756	29.116	31.232	133 LEU	C	12.393	25.365	45.081
123 SER	CB	15.184	28.969	29.748	133 LEU	O	13.539	25.053	44.693

FIGURE 1

134 GLU	N	12.164	26.195	46.111	143 ARG	NE	22.030	17.901	45.589
134 GLU	CA	13.276	26.827	46.768	143 ARG	CZ	21.037	17.422	44.816
134 GLU	CB	12.749	27.786	47.793	143 ARG	NH1	20.140	18.281	44.355
134 GLU	CG	13.795	28.476	48.645	143 ARG	NH2	20.887	16.134	44.524
134 GLU	CD	13.249	29.330	49.814	143 ARG	C	26.745	20.456	44.602
134 GLU	OK1	14.013	30.014	50.482	143 ARG	O	27.216	19.708	43.740
134 GLU	OK2	12.046	29.337	50.036	144 GLY	N	27.007	21.760	44.635
134 GLU	C	14.181	25.795	47.420	144 GLY	CA	27.925	22.456	43.737
134 GLU	O	15.396	25.915	47.353	144 GLY	C	27.365	22.887	42.396
135 GLN	N	13.598	24.770	48.060	144 GLY	O	28.139	23.324	41.539
135 GLN	CA	14.373	23.701	48.651	145 VAL	N	26.048	22.782	42.186
135 GLN	CB	13.350	22.830	49.331	145 VAL	CA	25.465	23.150	40.874
135 GLN	CG	13.897	21.596	50.006	145 VAL	CB	24.118	22.435	40.672
135 GLN	CD	12.823	20.790	50.764	145 VAL	CG1	23.521	22.778	39.291
135 GLN	OK1	11.779	20.305	50.258	145 VAL	CG2	24.324	20.921	40.792
135 GLN	NE2	13.143	20.692	52.060	145 VAL	C	25.262	24.680	40.827
135 GLN	C	15.248	22.952	47.620	145 VAL	O	24.836	25.282	41.840
135 GLN	O	16.434	22.651	47.868	146 LEU	N	25.677	25.350	39.742
136 ALA	N	14.690	22.749	46.420	146 LEU	CA	25.317	26.759	39.578
136 ALA	CA	15.406	22.071	45.337	146 LEU	CB	26.351	27.518	38.740
136 ALA	CB	14.430	21.762	44.225	146 LEU	CG	26.005	28.987	38.374
136 ALA	C	16.556	22.950	44.802	146 LEU	CD1	25.819	29.816	39.604
136 ALA	O	17.676	22.465	44.513	146 LEU	CD2	27.114	29.556	37.506
137 VAL	N	16.313	24.272	44.677	146 LEU	C	23.979	26.800	38.875
137 VAL	CA	17.375	25.224	44.305	146 LEU	O	23.873	26.371	37.710
137 VAL	CB	16.834	26.694	44.238	147 VAL	N	22.940	27.297	39.523
137 VAL	CG1	17.998	27.738	44.134	147 VAL	CA	21.611	27.371	38.926
137 VAL	CG2	15.876	26.776	43.047	147 VAL	CB	20.552	27.093	40.011
137 VAL	C	18.531	25.152	45.317	147 VAL	CG1	19.153	27.272	39.387
137 VAL	O	19.711	24.982	44.974	147 VAL	CG2	20.649	25.642	40.526
138 ASN	N	18.136	25.179	46.588	147 VAL	C	21.405	28.740	38.305
138 ASN	CA	19.136	25.146	47.616	147 VAL	O	21.480	29.768	38.965
138 ASN	CB	18.498	25.457	48.973	148 VAL	N	21.138	28.776	37.003
138 ASN	CG	18.125	26.934	49.063	148 VAL	CA	21.007	30.019	36.251
138 ASN	OD1	18.598	27.789	48.320	148 VAL	CB	21.982	30.003	35.055
138 ASN	ND2	17.258	27.299	49.985	148 VAL	CG1	21.916	31.349	34.328
138 ASN	C	19.869	23.832	47.685	148 VAL	CG2	23.403	29.791	35.562
138 ASN	O	21.103	23.849	47.846	148 VAL	C	19.557	30.040	35.781
139 SER	N	19.209	22.709	47.506	148 VAL	O	19.127	29.064	35.128
139 SER	CA	19.937	21.466	47.610	149 ALA	N	18.826	31.120	36.019
139 SER	CB	19.001	20.303	47.649	149 ALA	CA	17.387	31.187	35.758
139 SER	CG	18.203	20.407	46.479	149 ALA	CB	16.610	31.028	37.063
139 SER	C	20.860	21.316	46.403	149 ALA	C	16.952	32.515	35.111
139 SER	O	22.027	20.902	46.586	149 ALA	O	17.539	33.555	35.396
140 ALA	N	20.431	21.663	45.160	150 ALA	N	15.931	32.454	34.249
140 ALA	CA	21.392	21.545	44.053	150 ALA	CA	15.375	33.605	33.549
140 ALA	CB	20.755	21.895	42.723	150 ALA	CB	14.427	33.109	32.448
140 ALA	C	22.593	22.460	44.264	150 ALA	C	14.588	34.558	34.469
140 ALA	O	23.740	22.070	44.057	150 ALA	O	13.789	34.092	35.290
141 THR	N	22.377	23.682	44.756	151 SER	N	14.717	35.878	34.313
141 THR	CA	23.473	24.599	45.081	151 SER	CA	13.991	36.841	35.145
141 THR	CB	22.851	25.918	45.587	151 SER	CB	14.526	38.284	34.979
141 THR	CG1	22.034	26.472	44.549	151 SER	OG	14.430	38.730	33.630
141 THR	CG2	23.908	26.914	45.924	151 SER	C	12.485	36.873	34.867
141 THR	C	24.419	23.994	46.121	151 SER	O	11.692	37.218	35.761
141 THR	O	25.644	24.054	45.907	152 GLY	N	12.062	36.534	33.633
142 SER	N	23.975	23.363	47.202	152 GLY	CA	10.646	36.425	33.269
142 SER	CA	24.937	22.839	48.134	152 GLY	C	10.382	37.457	32.193
142 SER	CB	24.216	22.599	49.442	152 GLY	O	11.117	38.447	32.024
142 SER	CG	23.086	21.786	49.207	153 ASN	N	9.271	37.263	31.499
142 SER	C	25.620	21.592	47.583	153 ASN	CA	8.969	38.082	30.352
142 SER	O	26.616	21.131	48.150	153 ASN	CB	8.689	37.237	29.116
143 ARG	N	25.155	21.025	46.447	153 ASN	CG	9.865	36.443	28.658
143 ARG	CA	25.865	19.945	45.761	153 ASN	OD1	11.041	36.707	28.880
143 ARG	CB	24.848	18.907	45.261	153 ASN	ND2	9.501	35.336	27.944
143 ARG	CG	24.269	18.107	46.467	153 ASN	C	7.759	38.990	30.526
143 ARG	CD	23.132	17.127	46.152	153 ASN	O	7.190	39.421	29.524

SUBSTITUTE SHEET

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FIGURE 1

154	SER	N	7.390	39.398	31.739	164	ARG	CB	12.939	36.127	43.071
154	SER	CA	6.193	40.206	31.915	164	ARG	CG	12.741	37.084	44.237
154	SER	CB	5.577	39.973	33.284	164	ARG	CD	13.377	38.408	43.906
154	SER	OG	6.365	40.558	34.319	164	ARG	NE	13.251	39.367	44.988
154	SER	C	6.534	41.682	31.798	164	ARG	CZ	14.206	39.530	45.901
154	SER	O	5.599	42.468	31.793	164	ARG	NH1	14.020	40.475	46.838
155	GLY	N	7.805	42.092	31.773	164	ARG	NH2	15.289	38.737	45.965
155	GLY	CA	8.154	43.499	31.759	164	ARG	C	15.032	35.123	43.973
155	GLY	C	8.028	44.150	33.143	164	ARG	O	15.559	35.875	44.807
155	GLY	O	8.292	45.349	33.278	165	TYR	N	15.147	33.808	44.046
156	ALA	N	7.640	43.439	34.195	165	TYR	CA	15.787	33.157	45.176
156	ALA	CA	7.476	44.065	35.498	165	TYR	CB	15.503	31.609	45.150
156	ALA	CB	6.649	43.170	36.405	165	TYR	CG	14.046	31.230	45.501
156	ALA	C	8.814	44.359	36.187	165	TYR	CD1	13.399	31.807	46.600
156	ALA	O	9.864	43.754	35.891	165	TYR	CE1	12.084	31.484	46.885
157	SER	N	8.746	45.315	37.132	165	TYR	CD2	13.379	30.328	44.696
157	SER	CA	9.857	45.747	37.932	165	TYR	CE2	12.067	30.003	44.992
157	SER	CB	9.592	47.150	38.402	165	TYR	CZ	11.444	30.587	46.078
157	SER	OG	8.442	47.158	39.213	165	TYR	OH	10.133	30.227	46.357
157	SER	C	10.085	44.828	39.123	165	TYR	C	17.293	33.408	45.179
157	SER	O	10.623	45.251	40.147	165	TYR	O	17.996	33.477	44.141
158	SER	N	9.695	43.568	39.049	166	ALA	N	17.829	33.600	46.368
158	SER	CA	10.126	42.600	40.061	166	ALA	CA	19.222	33.986	46.544
158	SER	CB	9.046	42.518	41.150	166	ALA	CB	19.552	34.070	48.042
158	SER	OG	7.823	41.997	40.640	166	ALA	C	20.231	33.070	45.878
158	SER	C	10.335	41.293	39.275	166	ALA	O	21.192	33.553	45.278
158	SER	O	9.682	41.091	38.225	167	ASN	N	19.920	31.767	45.871
159	ILE	N	11.265	40.413	39.718	167	ASN	CA	20.860	30.806	45.280
159	ILE	CA	11.600	39.245	38.894	167	ASN	CB	20.778	29.446	46.048
159	ILE	CB	13.164	39.024	38.847	167	ASN	CG	21.566	29.545	47.374
159	ILE	CG2	13.801	40.300	38.272	167	ASN	OD1	22.592	30.238	47.502
159	ILE	CG1	13.729	38.612	40.201	167	ASN	ND2	21.130	28.931	48.461
159	ILE	CD	15.208	38.246	40.013	167	ASN	C	20.712	30.572	43.776
159	ILE	C	10.906	37.978	39.381	167	ASN	O	21.411	29.727	43.205
159	ILE	O	10.454	37.888	40.528	168	ALA	N	19.760	31.248	43.121
160	SER	N	10.806	36.974	38.510	168	ALA	CA	19.673	31.167	41.683
160	SER	CA	10.114	35.754	38.841	168	ALA	CB	18.206	31.007	41.284
160	SER	CB	9.658	35.097	37.513	168	ALA	C	20.259	32.481	41.121
160	SER	OG	10.700	34.817	36.581	168	ALA	O	19.961	33.600	41.595
160	SER	C	10.947	34.777	39.691	169	MET	N	21.005	32.366	40.015
160	SER	O	12.152	34.921	39.958	169	MET	CA	21.563	33.497	39.321
161	TYR	N	10.265	33.738	40.148	169	MET	CB	22.854	33.069	38.636
161	TYR	CA	10.867	32.645	40.876	169	MET	CG	23.476	34.273	37.972
161	TYR	CB	9.887	32.231	41.988	169	MET	SD	25.057	33.851	37.212
161	TYR	CG	9.698	33.315	43.030	169	MET	CE	25.641	35.532	37.199
161	TYR	CD1	10.614	33.397	44.072	169	MET	C	20.493	33.939	38.305
161	TYR	CE1	10.459	34.368	45.057	169	MET	O	19.998	33.150	37.484
161	TYR	CD2	8.619	34.189	42.939	170	ALA	N	20.047	35.196	38.436
161	TYR	CE2	8.459	35.175	43.906	170	ALA	CA	18.956	35.777	37.681
161	TYR	CZ	9.384	35.241	44.953	170	ALA	CB	18.208	36.758	38.591
161	TYR	OH	9.270	36.241	45.896	170	ALA	C	19.430	36.504	36.432
161	TYR	C	11.101	31.499	39.865	170	ALA	O	20.278	37.405	36.596
161	TYR	O	10.257	31.307	38.975	171	VAL	N	18.927	36.158	35.241
162	PRO	N	12.153	30.681	39.954	171	VAL	CA	19.332	36.739	33.966
162	PRO	CD	12.388	29.536	39.042	171	VAL	CB	19.862	35.590	33.075
162	PRO	CA	13.162	30.687	41.003	171	VAL	CG1	20.380	36.267	31.766
162	PRO	CB	13.715	29.232	40.966	171	VAL	CG2	20.946	34.749	33.786
162	PRO	CG	13.726	28.915	39.470	171	VAL	C	18.192	37.445	33.235
162	PRO	C	14.243	31.756	40.879	171	VAL	O	17.145	36.824	32.979
162	PRO	O	15.044	31.845	41.789	172	GLY	N	18.474	38.712	32.887
163	ALA	N	14.352	32.580	39.814	172	GLY	CA	17.594	39.568	32.123
163	ALA	CA	15.393	33.575	39.716	172	GLY	C	18.038	39.553	30.640
163	ALA	CB	15.165	34.416	38.441	172	GLY	O	19.106	39.023	30.302
163	ALA	C	15.538	34.529	40.935	173	ALA	N	17.231	40.184	29.781
163	ALA	O	16.640	34.874	41.399	173	ALA	CA	17.461	40.220	28.347
164	ARG	N	14.417	34.878	41.559	173	ALA	CB	16.278	39.626	27.617
164	ARG	CA	14.385	35.740	42.745	173	ALA	C	17.667	41.631	27.812

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FIGURE 1

173	ALA	O	16.987	42.599	28.169	182	SER	CB	9.735	46.338	25.967
174	THR	N	18.639	41.714	26.910	182	SER	OG	9.316	45.472	24.927
174	THR	CA	18.993	42.944	26.178	182	SER	C	10.061	44.602	27.814
174	THR	CB	20.504	43.281	26.349	182	SER	O	9.557	44.957	28.879
174	THR	OG1	21.329	42.130	26.074	183	PHE	N	10.058	43.321	27.403
174	THR	CG2	20.733	43.727	27.767	183	PHE	CA	9.501	42.231	28.191
174	THR	C	18.715	42.787	24.688	183	PHE	CB	9.261	40.999	27.282
174	THR	O	18.622	41.662	24.148	183	PHE	CG	10.501	40.486	26.518
175	ASP	N	18.674	43.934	24.029	183	PHE	CD1	11.508	39.734	27.142
175	ASP	CA	18.518	43.907	22.588	183	PHE	CD2	10.653	40.840	25.173
175	ASP	CB	17.388	44.840	22.148	183	PHE	CE1	12.617	39.325	26.421
175	ASP	CG	17.584	46.353	22.386	183	PHE	CE2	11.782	40.415	24.478
175	ASP	OD1	18.675	46.844	22.682	183	PHE	CZ	12.774	39.665	25.095
175	ASP	OD2	16.579	47.047	22.291	183	PHE	C	10.359	41.795	29.380
175	ASP	C	19.794	44.258	21.834	183	PHE	O	9.889	41.025	30.246
175	ASP	O	20.844	44.480	22.440	184	SER	N	11.615	42.247	29.427
176	GLN	N	19.724	44.498	20.516	184	SER	CA	12.551	41.670	30.410
176	GLN	CA	20.938	44.742	19.737	184	SER	CB	13.998	42.030	30.045
176	GLN	CB	20.702	44.722	18.237	184	SER	OG	14.926	41.420	30.947
176	GLN	CG	20.123	43.400	17.797	184	SER	C	12.281	42.125	31.843
176	GLN	CD	18.592	43.272	17.887	184	SER	O	12.450	43.331	32.137
176	GLN	OE1	17.837	44.022	18.543	185	GLN	N	11.911	41.197	32.727
176	GLN	NE2	18.083	42.254	17.196	185	GLN	CA	11.652	41.622	34.089
176	GLN	C	21.534	46.084	20.056	185	GLN	CB	11.034	40.489	34.904
176	GLN	O	22.690	46.302	19.783	185	GLN	CG	9.595	40.335	34.482
177	ASN	N	20.836	46.989	20.719	185	GLN	CD	8.912	39.174	35.165
177	ASN	CA	21.382	48.288	21.098	185	GLN	OE1	8.817	39.005	36.377
177	ASN	CB	20.321	49.300	20.975	185	GLN	NE2	8.397	38.320	34.331
177	ASN	CG	19.832	49.550	19.587	185	GLN	C	12.960	42.075	34.773
177	ASN	OD1	20.577	49.605	18.631	185	GLN	O	14.066	41.606	34.458
177	ASN	ND2	18.526	49.678	19.484	186	TYR	N	12.871	43.046	35.676
177	ASN	C	21.895	48.299	22.521	186	TYR	CA	14.048	43.618	36.349
177	ASN	O	22.380	49.322	23.026	186	TYR	CB	14.488	44.924	35.634
178	ASN	N	21.875	47.139	23.202	186	TYR	CG	13.385	45.992	35.576
178	ASN	CA	22.256	47.033	24.623	186	TYR	CD1	12.362	45.872	34.635
178	ASN	CB	23.735	47.479	24.896	186	TYR	CE1	11.347	46.805	34.553
178	ASN	CG	24.734	46.515	24.314	186	TYR	CD2	13.385	47.049	36.468
178	ASN	OD1	24.433	45.324	24.210	186	TYR	CE2	12.386	47.988	36.396
178	ASN	ND2	25.920	46.928	23.917	186	TYR	CZ	11.376	47.855	35.450
178	ASN	C	21.345	47.835	25.547	186	TYR	OH	10.418	48.846	35.328
178	ASN	O	21.747	48.392	26.576	186	TYR	C	13.735	43.925	37.819
179	ASN	N	20.081	47.806	25.174	186	TYR	O	12.616	43.620	38.262
179	ASN	CA	19.000	48.319	26.009	187	GLY	N	14.620	44.547	38.575
179	ASN	CB	18.044	49.165	25.243	187	GLY	CA	14.330	44.849	39.958
179	ASN	CG	18.566	50.593	25.088	187	GLY	C	15.232	44.062	40.892
179	ASN	OD1	19.289	51.155	25.949	187	GLY	O	16.318	43.548	40.541
179	ASN	ND2	18.250	51.181	23.925	188	ALA	N	14.782	43.915	42.140
179	ASN	C	18.230	47.101	26.490	188	ALA	CA	15.616	43.340	43.172
179	ASN	O	18.246	46.016	25.872	188	ALA	CB	14.891	43.435	44.515
180	ARG	N	17.579	47.276	27.645	188	ALA	C	15.973	41.884	42.894
180	ARG	CA	16.734	46.241	28.230	188	ALA	O	15.134	41.065	42.549
180	ARG	CB	16.050	46.746	29.525	189	GLY	N	17.263	41.594	42.986
180	ARG	CG	15.269	45.653	30.233	189	GLY	CA	17.747	40.223	42.778
180	ARG	CD	14.562	46.201	31.492	189	GLY	C	18.299	39.938	41.358
180	ARG	NE	13.537	47.146	31.076	189	GLY	O	18.911	38.873	41.139
180	ARG	CZ	12.271	46.850	30.720	190	LEU	N	18.128	40.857	40.397
180	ARG	NH1	11.476	47.846	30.339	190	LEU	CA	18.646	40.601	39.064
180	ARG	NH2	11.709	45.650	30.752	190	LEU	CB	18.023	41.621	38.094
180	ARG	C	15.639	45.909	27.213	190	LEU	CG	18.302	41.454	36.607
180	ARG	O	14.991	46.855	26.715	190	LEU	CD1	17.688	40.163	36.140
181	ALA	N	15.377	44.644	26.848	190	LEU	CD2	17.844	42.716	35.848
181	ALA	CA	14.225	44.338	26.002	190	LEU	C	20.169	40.671	39.079
181	ALA	CB	14.266	42.883	25.663	190	LEU	O	20.776	41.624	39.589
181	ALA	C	12.942	44.677	26.771	191	ASP	N	20.847	39.677	38.505
181	ALA	O	12.873	44.495	28.009	191	ASP	CA	22.285	39.597	38.558
182	SER	N	11.894	45.172	26.133	191	ASP	CB	22.732	38.163	28.777
182	SER	CA	10.757	45.650	26.927	191	ASP	CG	22.428	37.668	40.182

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FIGURE 1

191 ASP OD1	22.903	38.250	41.148	201 SER CA	19.476	28.584	17.356
191 ASP OD2	21.685	36.717	40.309	201 SER CB	19.283	28.528	18.891
191 ASP C	23.037	40.095	37.355	201 SER OG	20.089	27.563	19.530
191 ASP O	24.122	40.674	37.449	201 SER C	18.875	27.346	16.701
192 ILE N	22.464	39.842	36.171	201 SER O	18.062	27.448	15.779
192 ILE CA	23.192	40.070	34.908	202 THR N	19.318	26.189	17.171
192 ILE CB	24.291	38.919	34.852	202 THR CA	18.879	24.880	16.747
192 ILE CG2	23.628	37.619	34.325	202 THR CB	19.769	23.760	17.461
192 ILE CG1	25.513	39.314	34.012	202 THR OG1	19.869	24.043	18.866
192 ILE CD	26.686	38.323	34.226	202 THR CG2	21.204	23.712	16.888
192 ILE C	22.176	40.008	33.774	202 THR C	17.412	24.706	17.082
192 ILE O	21.020	39.545	33.967	202 THR O	16.901	25.159	18.115
193 VAL N	22.644	40.477	32.608	203 TYR N	16.712	23.986	16.227
193 VAL CA	21.847	40.379	31.392	203 TYR CA	15.286	23.728	16.398
193 VAL CB	21.246	41.745	30.945	203 TYR CB	14.508	24.820	15.615
193 VAL CG1	20.189	42.187	31.967	203 TYR CG	13.165	25.140	16.239
193 VAL CG2	22.326	42.772	30.755	203 TYR CD1	13.129	25.884	17.421
193 VAL C	22.653	39.820	30.203	203 TYR CE1	11.918	26.223	17.992
193 VAL O	23.885	39.799	30.174	203 TYR CD2	11.996	24.708	15.619
194 ALA N	21.891	39.376	29.204	203 TYR CE2	10.770	25.044	16.193
194 ALA CA	22.453	38.810	28.000	203 TYR CZ	10.757	25.798	17.369
194 ALA CB	22.770	37.303	28.253	203 TYR OH	9.560	26.166	17.949
194 ALA C	21.446	38.965	26.837	203 TYR C	14.941	22.322	15.901
194 ALA O	20.264	39.273	27.044	203 TYR O	15.658	21.779	15.040
195 PRO N	21.872	38.794	25.576	204 PRO N	13.905	21.662	16.450
195 PRO CD	23.294	38.583	25.188	204 PRO CD	13.057	22.111	17.596
195 PRO CA	21.018	38.880	24.377	204 PRO CA	13.468	20.319	15.980
195 PRO CB	21.899	38.465	23.180	204 PRO CB	12.178	20.026	16.797
195 PRO CG	23.321	38.854	23.643	204 PRO CG	12.414	20.819	18.098
195 PRO C	19.802	38.002	24.479	204 PRO C	13.249	20.306	14.463
195 PRO O	19.931	36.816	24.761	204 PRO O	12.965	21.337	13.825
196 GLY N	18.648	38.574	24.192	205 GLY N	13.473	19.119	13.895
196 GLY CA	17.403	37.833	24.257	205 GLY CA	13.358	18.927	12.435
196 GLY C	16.401	38.217	23.175	205 GLY C	14.643	19.310	11.724
196 GLY O	15.214	37.925	23.303	205 GLY O	14.632	19.630	10.535
197 VAL N	16.829	38.890	22.088	206 SER N	15.770	19.252	12.442
197 VAL CA	15.888	39.285	21.035	206 SER CA	17.067	19.586	11.924
197 VAL CB	15.690	40.877	21.010	206 SER CB	17.523	18.417	11.036
197 VAL CG1	14.919	41.323	19.738	206 SER OG	17.461	17.216	11.797
197 VAL CG2	15.038	41.327	22.327	206 SER C	17.098	20.931	11.175
197 VAL C	16.483	38.785	19.727	206 SER O	17.591	21.045	10.047
197 VAL O	17.672	38.897	19.432	207 THR N	16.566	21.968	11.842
198 ASN N	15.627	38.173	18.937	207 THR CA	16.518	23.294	11.258
198 ASN CA	15.957	37.626	17.630	207 THR CB	15.070	23.518	10.667
198 ASN CB	16.220	38.703	16.520	207 THR OG1	15.190	24.695	9.866
198 ASN CG	15.814	38.095	15.160	207 THR CG2	13.924	23.606	11.700
198 ASN OD1	15.010	37.149	15.093	207 THR C	16.928	24.275	12.354
198 ASN ND2	16.255	38.621	14.013	207 THR O	17.600	23.908	13.342
198 ASN C	17.160	36.718	17.695	208 TYR N	16.632	25.546	12.113
198 ASN O	18.147	36.910	16.978	208 TYR CA	17.071	26.693	12.914
199 VAL N	17.039	35.746	18.605	208 TYR CB	18.333	27.321	12.307
199 VAL CA	18.096	34.791	18.849	208 TYR CG	19.364	26.245	12.061
199 VAL CB	18.135	34.490	20.377	208 TYR CD1	19.428	25.565	10.842
199 VAL CG1	19.303	33.623	20.702	208 TYR CE1	20.274	24.513	10.648
199 VAL CG2	18.493	35.732	21.205	208 TYR CD2	20.152	25.869	13.110
199 VAL C	17.872	33.522	18.017	208 TYR CE2	20.978	24.825	12.917
199 VAL O	16.912	32.776	18.194	208 TYR CZ	21.039	24.151	11.713
200 GLN N	18.706	33.324	17.005	208 TYR OH	21.935	23.103	11.601
200 GLN CA	18.771	32.144	16.138	208 TYR C	15.936	27.689	12.911
200 GLN CB	19.584	32.515	14.908	208 TYR O	15.224	27.863	11.906
200 GLN CG	19.819	31.348	13.964	209 ALA N	15.728	28.316	14.076
200 GLN CD	20.240	31.677	12.544	209 ALA CA	14.653	29.234	14.266
200 GLN OE1	21.324	32.176	12.338	209 ALA CB	13.489	28.384	14.707
200 GLN NE2	19.502	31.494	11.476	209 ALA C	15.041	30.312	15.266
200 GLN C	19.433	30.946	16.796	209 ALA O	16.021	30.178	16.019
200 GLN O	20.567	31.114	17.277	210 SER N	14.378	31.450	15.089
201 SER N	18.810	29.769	16.799	210 SER CA	14.567	32.642	15.914

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FIGURE 1

210	SER	CB	14.614	33.893	15.065	220	HIS	CG	23.307	34.345	25.237
210	SER	OG	15.788	33.756	14.342	220	HIS	CD2	24.010	34.501	24.048
210	SER	C	13.456	32.819	16.920	220	HIS	ND1	21.999	34.359	24.936
210	SER	O	12.255	32.689	16.610	220	HIS	CE1	21.849	34.518	23.642
211	LEU	N	13.895	33.079	18.152	220	HIS	NE2	23.064	34.607	23.115
211	LEU	CA	12.990	33.304	19.244	220	HIS	C	25.048	32.824	28.410
211	LEU	CB	12.963	32.089	20.118	220	HIS	O	26.276	32.626	28.383
211	LEU	CG	12.368	30.848	19.535	221	VAL	N	24.370	32.933	29.566
211	LEU	CD1	12.346	29.857	20.657	221	VAL	CA	25.084	32.989	30.830
211	LEU	CD2	10.940	31.056	19.033	221	VAL	CB	24.180	33.727	31.843
211	LEU	C	13.372	34.503	20.110	221	VAL	CG1	24.746	33.674	33.267
211	LEU	O	14.547	34.927	20.110	221	VAL	CG2	24.119	35.194	31.366
212	ASN	N	12.439	35.024	20.912	221	VAL	C	25.477	31.606	31.299
212	ASN	CA	12.734	36.191	21.741	221	VAL	O	26.612	31.424	31.734
212	ASN	CB	11.883	37.403	21.413	222	ALA	N	24.617	30.614	31.120
212	ASN	CG	11.961	37.853	19.972	222	ALA	CA	24.981	29.223	31.421
212	ASN	OD1	12.979	38.246	19.415	222	ALA	CB	23.871	28.283	31.032
212	ASN	ND2	10.841	37.797	19.283	222	ALA	C	26.229	28.786	30.670
212	ASN	C	12.354	35.787	23.156	222	ALA	O	27.129	28.121	31.204
212	ASN	O	11.336	35.119	23.350	223	GLY	N	26.258	29.180	29.388
213	GLY	N	13.070	36.197	24.217	223	GLY	CA	27.463	28.928	28.649
213	GLY	CA	12.648	35.928	25.599	223	GLY	C	28.715	29.661	29.070
213	GLY	C	13.834	35.974	26.520	223	GLY	O	29.806	29.064	29.098
213	GLY	O	14.990	35.843	26.099	224	ALA	N	28.557	30.955	29.357
214	THR	N	13.583	36.141	27.832	224	ALA	CA	29.708	31.677	29.842
214	THR	CA	14.658	36.016	28.829	224	ALA	CB	29.313	33.106	30.058
214	THR	CB	14.204	36.523	30.242	224	ALA	C	30.261	31.051	31.147
214	THR	OG1	12.998	35.812	30.594	224	ALA	O	31.463	30.894	31.314
214	THR	CG2	14.014	38.055	30.271	225	ALA	N	29.387	30.580	32.016
214	THR	C	15.128	34.527	28.894	225	ALA	CA	29.771	29.836	33.221
214	THR	O	16.253	34.214	29.302	225	ALA	CB	28.560	29.321	34.020
215	SER	N	14.304	33.607	28.380	225	ALA	C	30.593	28.603	32.864
215	SER	CA	14.663	32.187	28.217	225	ALA	O	31.630	28.374	33.487
215	SER	CB	13.425	31.449	27.696	226	ALA	N	30.248	27.816	31.843
215	SER	OG	12.324	31.235	28.564	226	ALA	CA	31.033	26.664	31.490
215	SER	C	15.860	31.981	27.237	226	ALA	CB	30.292	25.958	30.380
215	SER	O	16.588	30.993	27.305	226	ALA	C	32.446	27.078	31.054
216	MET	N	16.039	32.907	26.272	226	ALA	O	33.421	26.381	31.370
216	MET	CA	17.165	32.901	25.324	227	LEU	N	32.587	28.209	30.328
216	MET	CB	16.776	33.575	24.055	227	LEU	CA	33.888	28.734	29.901
216	MET	CG	15.843	32.791	23.121	227	LEU	CB	33.691	29.983	28.955
216	MET	SD	14.133	32.519	23.660	227	LEU	CG	32.901	29.762	27.666
216	MET	CE	14.311	30.783	23.925	227	LEU	CD1	32.816	31.015	26.813
216	MET	C	18.372	33.638	25.885	227	LEU	CD2	33.598	28.704	26.902
216	MET	O	19.506	33.386	25.460	227	LEU	C	34.782	29.060	31.088
217	ALA	N	18.136	34.558	26.845	227	LEU	O	35.954	28.623	31.131
217	ALA	CA	19.249	35.257	27.465	228	VAL	N	34.176	29.711	32.105
217	ALA	CB	18.739	36.485	28.240	228	VAL	CA	34.951	30.076	33.286
217	ALA	C	19.991	34.343	28.432	228	VAL	CB	34.114	31.094	34.168
217	ALA	O	21.223	34.249	28.386	228	VAL	CG1	34.822	31.451	35.502
218	THR	N	19.211	33.574	29.199	228	VAL	CG2	33.950	32.402	33.362
218	THR	CA	19.756	32.657	30.231	228	VAL	C	35.340	28.814	34.074
218	THR	CB	18.587	31.860	30.888	228	VAL	O	36.468	28.777	34.573
218	THR	OG1	17.719	32.837	31.429	229	LYS	N	34.502	27.781	34.115
218	THR	CG2	19.040	30.887	31.979	229	LYS	CA	34.817	26.566	34.865
218	THR	C	20.824	31.704	29.700	229	LYS	CB	33.575	25.679	34.978
218	THR	O	21.912	31.648	30.275	229	LYS	CG	33.758	24.324	35.713
219	PRO	N	20.683	31.008	28.586	229	LYS	CD	34.180	24.479	37.170
219	PRO	CD	19.479	30.843	27.793	229	LYS	CE	34.230	23.097	37.844
219	PRO	CA	21.708	30.099	28.089	229	LYS	NZ	34.394	23.211	39.298
219	PRO	CB	21.074	29.384	26.909	229	LYS	C	35.919	25.792	34.170
219	PRO	CG	19.943	30.268	26.471	229	LYS	O	36.804	25.233	34.841
219	PRO	C	23.027	30.765	27.704	230	GLN	N	35.915	25.679	32.835
219	PRO	O	24.060	30.108	27.745	230	GLN	CA	37.001	24.957	32.188
220	HIS	K	22.994	32.051	27.345	230	GLN	CB	36.692	24.852	30.683
220	HIS	CA	24.239	32.770	27.094	230	GLN	CG	37.819	24.181	29.916
220	HIS	CB	23.997	34.219	26.600	230	GLN	CD	37.806	24.343	28.410

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FIGURE 1

230 GLN OE1	36.941	24.907	27.731	238 VAL C	30.741	31.770	43.322
230 GLN NE2	38.866	23.779	27.864	238 VAL O	30.584	32.955	42.971
230 GLN C	38.324	25.710	32.453	239 GLN N	31.903	31.146	43.181
230 GLN O	39.365	25.106	32.722	239 GLN CA	33.058	31.865	42.654
231 LYS N	38.320	27.043	32.369	239 GLN CB	34.348	31.007	42.712
231 LYS CA	39.482	27.877	32.678	239 GLN CG	34.787	30.771	44.165
231 LYS CB	39.085	29.347	32.389	239 GLN CD	36.001	29.847	44.293
231 LYS CG	40.041	30.518	32.637	239 GLN OE1	35.946	28.629	44.354
231 LYS CD	41.380	30.478	31.945	239 GLN NE2	37.174	30.441	44.326
231 LYS CE	42.078	31.872	31.997	239 GLN C	32.811	32.264	41.203
231 LYS NZ	42.377	32.352	33.343	239 GLN O	33.124	33.398	40.784
231 LYS C	39.970	27.715	34.142	240 ILE N	32.261	31.291	40.463
231 LYS O	41.173	27.658	34.409	240 ILE CA	31.950	31.500	39.047
232 ASN N	39.023	27.635	35.097	240 ILE CB	31.410	30.186	38.368
232 ASN CA	39.292	27.588	36.520	240 ILE CG2	31.025	30.399	36.876
232 ASN CB	38.801	28.848	37.227	240 ILE CG1	32.503	29.161	38.463
232 ASN CG	39.339	30.115	36.617	240 ILE CD	32.041	27.775	37.973
232 ASN OD1	40.486	30.464	36.859	240 ILE C	30.902	32.584	38.896
232 ASN ND2	38.537	30.834	35.845	240 ILE O	31.087	33.511	38.104
232 ASN C	38.595	26.402	37.158	241 ARG N	29.819	32.484	39.667
232 ASN O	37.635	26.555	37.907	241 ARG CA	28.769	33.495	39.638
233 PRO N	39.057	25.173	36.945	241 ARG CB	27.701	33.092	40.655
233 PRO CD	40.245	24.847	36.150	241 ARG CG	26.634	34.192	40.895
233 PRO CA	38.320	23.978	37.376	241 ARG CD	25.462	33.692	41.771
233 PRO CB	39.053	22.819	36.729	241 ARG NE	24.364	34.639	41.945
233 PRO CG	40.441	23.367	36.519	241 ARG CZ	23.323	34.340	42.749
233 PRO C	38.155	23.820	38.863	241 ARG NH1	22.325	35.215	42.920
233 PRO O	37.266	23.094	39.274	241 ARG NH2	23.252	33.149	43.371
234 SER N	38.962	24.489	39.675	241 ARG C	29.313	34.923	39.937
234 SER CA	38.725	24.374	41.124	241 ARG O	29.037	35.874	39.200
234 SER CB	40.005	24.643	41.961	242 ASN N	30.153	35.073	40.959
234 SER CG	40.378	26.007	41.847	242 ASN CA	30.649	36.413	41.277
234 SER C	37.635	25.309	41.680	242 ASN CB	31.391	36.455	42.609
234 SER O	37.203	25.124	42.824	242 ASN CG	30.386	36.371	43.746
235 TRP N	37.151	26.270	40.878	242 ASN OD1	29.177	36.652	43.659
235 TRP CA	36.213	27.246	41.393	242 ASN ND2	30.877	35.881	44.877
235 TRP CB	36.022	28.366	40.435	242 ASN C	31.591	36.931	40.225
235 TRP CG	37.165	29.323	40.391	242 ASN O	31.631	38.152	39.938
235 TRP CD2	37.103	30.539	39.761	243 HIS N	32.330	36.012	39.584
235 TRP CE2	38.384	31.011	39.929	243 HIS CA	33.284	36.451	38.593
235 TRP CE3	36.167	31.261	39.083	243 HIS CB	34.183	35.327	38.178
235 TRP CD1	38.405	29.059	40.930	243 HIS CG	35.409	35.790	37.413
235 TRP NE1	39.136	30.109	40.623	243 HIS CD2	36.367	36.638	37.902
235 TRP CZ2	38.726	32.237	39.404	243 HIS ND1	35.770	35.447	36.181
235 TRP CZ3	36.502	32.474	38.559	243 HIS CE1	36.908	36.044	35.892
235 TRP CH2	37.775	32.956	38.720	243 HIS NE2	37.250	36.757	36.945
235 TRP C	34.862	26.643	41.637	243 HIS C	32.559	36.966	37.370
235 TRP O	34.427	25.726	40.941	243 HIS O	32.988	37.984	36.820
236 SER N	34.206	27.137	42.669	244 LEU N	31.473	36.265	36.963
236 SER CA	32.884	26.712	43.011	244 LEU CA	30.709	36.649	35.801
236 SER CB	32.771	26.915	44.541	244 LEU CB	29.576	35.636	35.501
236 SER CG	32.691	28.301	44.902	244 LEU CG	29.971	34.234	34.958
236 SER C	31.891	27.549	42.200	244 LEU CD1	28.719	33.367	34.841
236 SER O	32.195	28.606	41.637	244 LEU CD2	30.649	34.360	33.602
237 ASN N	30.645	27.084	42.278	244 LEU C	30.147	38.007	36.104
237 ASN CA	29.495	27.743	41.705	244 LEU O	30.189	38.853	35.217
237 ASN CB	28.255	26.923	42.112	245 LYS N	29.690	38.289	37.328
237 ASN CG	27.966	26.679	43.605	245 LYS CA	29.178	39.632	37.654
237 ASN OD1	28.706	27.112	44.495	245 LYS CB	28.452	39.593	38.993
237 ASN ND2	26.851	26.017	43.928	245 LYS CG	27.193	38.687	38.928
237 ASN C	29.388	29.219	42.117	245 LYS CD	26.536	38.412	40.289
237 ASN O	29.255	30.109	41.266	245 LYS CE	25.811	39.677	40.573
238 VAL N	29.592	29.555	43.414	245 LYS NZ	25.221	39.607	41.886
238 VAL CA	29.576	30.945	43.876	245 LYS C	30.300	40.665	37.714
238 VAL CB	29.553	30.919	45.442	245 LYS O	30.125	41.805	37.257
238 VAL CG1	29.767	32.294	46.097	246 ASN N	31.452	40.279	38.195
238 VAL CG2	28.199	30.344	45.805	246 ASN CA	32.579	41.194	38.352

FIGURE 1

246	ASN	CB	33.697	40.568	39.196	256	LEU	CG	16.565	49.634	34.134
246	ASN	CG	33.286	40.502	40.651	256	LEU	CD1	16.919	50.482	32.887
246	ASN	OD1	32.445	41.245	41.165	256	LEU	CD2	15.095	49.321	34.182
246	ASN	ND2	33.814	39.538	41.375	256	LEU	C	18.284	46.162	34.546
246	ASN	C	33.188	41.620	37.046	256	LEU	O	17.798	45.300	33.803
246	ASN	O	33.819	42.686	36.960	257	TYR	N	19.566	46.246	34.888
247	THR	N	33.020	40.781	36.033	257	TYR	CA	20.590	45.390	34.268
247	THR	CA	33.574	41.115	34.734	257	TYR	CB	21.608	46.225	33.447
247	THR	CB	34.386	39.916	34.179	257	TYR	CG	20.957	47.106	32.389
247	THR	OG1	33.492	38.818	34.055	257	TYR	CD1	20.349	46.459	31.337
247	THR	CG2	35.608	39.565	35.059	257	TYR	CE1	19.733	47.179	30.384
247	THR	C	32.516	41.547	33.737	257	TYR	CD2	20.951	48.503	32.449
247	THR	O	32.865	41.792	32.575	257	TYR	CE2	20.330	49.219	31.446
248	ALA	N	31.252	41.714	34.123	257	TYR	CZ	19.731	48.536	30.426
248	ALA	CA	30.213	42.085	33.162	257	TYR	OH	19.142	49.131	29.335
248	ALA	CB	28.829	41.914	33.800	257	TYR	C	21.424	44.557	35.226
248	ALA	C	30.385	43.558	32.731	257	TYR	O	22.226	43.739	34.776
248	ALA	O	30.961	44.395	33.440	258	GLY	N	21.305	44.756	36.542
249	THR	N	29.950	43.949	31.551	258	GLY	CA	22.222	44.130	37.496
249	THR	CA	30.001	45.323	31.096	258	GLY	C	23.630	44.552	37.201
249	THR	CB	29.955	45.301	29.552	258	GLY	O	23.896	45.710	36.877
249	THR	OG1	31.151	44.706	29.080	259	SER	N	24.511	43.586	37.273
249	THR	CG2	29.830	46.690	28.965	259	SER	CA	25.897	43.856	36.955
249	THR	C	28.830	46.105	31.676	259	SER	CB	26.747	42.633	37.239
249	THR	O	27.664	45.760	31.425	259	SER	OG	26.779	42.518	38.660
250	SER	N	29.067	47.214	32.412	259	SER	C	26.153	44.278	35.527
250	SER	CA	27.941	47.994	32.947	259	SER	O	27.225	44.856	35.285
250	SER	CB	28.405	49.102	33.875	260	GLY	N	25.225	44.013	34.600
250	SER	OG	27.267	49.862	34.279	260	GLY	CA	25.413	44.431	33.222
250	SER	C	27.136	48.631	31.822	260	GLY	C	25.476	43.210	32.331
250	SER	O	27.687	49.164	30.857	260	GLY	O	24.999	42.106	32.672
251	LEU	N	25.824	48.523	31.929	261	LEU	N	26.036	43.461	31.151
251	LEU	CA	24.949	49.115	30.934	261	LEU	CA	26.105	42.461	30.087
251	LEU	CB	24.067	48.019	30.342	261	LEU	CB	26.274	43.195	28.721
251	LEU	CG	24.737	46.908	29.627	261	LEU	CG	26.349	42.381	27.424
251	LEU	CD1	23.663	46.020	29.043	261	LEU	CD1	25.064	41.598	27.191
251	LEU	CD2	25.595	47.430	28.481	261	LEU	CD2	26.675	43.372	26.282
251	LEU	C	24.069	50.231	31.462	261	LEU	C	27.234	41.470	30.309
251	LEU	O	23.214	50.787	30.769	261	LEU	O	28.410	41.842	30.426
252	GLY	N	24.239	50.606	32.703	262	VAL	N	26.851	40.192	30.263
252	GLY	CA	23.317	51.538	33.279	262	VAL	CA	27.872	39.161	30.432
252	GLY	C	22.880	50.976	34.613	262	VAL	CB	27.227	37.754	30.407
252	GLY	O	23.651	50.372	35.376	262	VAL	CG1	26.633	37.448	29.036
253	SER	N	21.614	51.241	34.872	262	VAL	CG2	28.305	36.734	30.824
253	SER	CA	20.958	50.918	36.106	262	VAL	C	28.935	39.300	29.331
253	SER	CB	19.470	51.165	35.891	262	VAL	O	28.661	39.699	28.193
253	SER	OG	18.813	51.273	37.150	263	ASN	N	30.181	39.070	29.700
253	SER	C	21.195	49.492	36.567	263	ASN	CA	31.271	39.216	28.755
253	SER	O	20.900	48.587	35.786	263	ASN	CB	31.866	40.599	28.993
254	THR	N	21.694	49.321	37.796	263	ASN	CG	33.072	40.880	28.136
254	THR	CA	21.773	48.021	38.431	263	ASN	OD1	33.666	40.009	27.502
254	THR	CB	22.417	48.071	39.869	263	ASN	ND2	33.498	42.124	28.143
254	THR	OG1	23.694	48.691	39.803	263	ASN	C	32.250	38.068	28.945
254	THR	CG2	22.671	46.670	40.414	263	ASN	O	33.119	37.994	29.826
254	THR	C	20.311	47.594	38.557	264	ALA	N	32.136	37.126	28.030
254	THR	O	20.041	46.419	38.445	264	ALA	CA	32.947	35.931	28.088
255	ASN	N	19.316	48.480	38.694	264	ALA	CB	32.528	34.857	27.080
255	ASN	CA	17.930	48.038	38.783	264	ALA	C	34.404	36.250	27.801
255	ASN	CB	17.061	49.253	39.031	264	ALA	O	35.259	35.517	28.331
255	ASN	CG	15.600	48.927	39.271	265	GLU	N	34.752	37.304	27.054
255	ASN	OD1	15.191	48.158	40.157	265	GLU	CA	36.169	37.625	26.884
255	ASN	ND2	14.771	49.580	38.459	265	GLU	CB	36.346	38.768	25.842
255	ASN	C	17.441	47.296	37.526	265	GLU	CG	37.790	39.302	25.597
255	ASN	O	16.752	46.279	37.550	265	GLU	CD	38.470	40.138	26.723
256	LEU	N	17.889	47.805	36.309	265	GLU	OE1	39.623	39.854	27.100
256	LEU	CA	17.437	47.297	35.108	265	GLD	OE2	37.835	41.060	27.255
256	LEU	CB	17.435	48.386	34.041	265	GLU	C	36.745	38.057	28.227

FIGURE 1

265	GLU	O	37.766	37.524	28.689	307	H2O	OH2	26.065	37.253	43.741
266	ALA	N	36.098	39.020	28.897	308	H2O	OH2	11.945	45.684	23.380
266	ALA	CA	36.698	39.536	30.109	309	H2O	OH2	19.643	10.507	40.112
266	ALA	CB	35.959	40.800	30.534	310	H2O	OH2	38.430	41.954	36.077
266	ALA	C	36.677	38.485	31.228	311	H2O	OH2	13.501	39.873	16.866
266	ALA	O	37.562	38.418	32.099	312	H2O	OH2	16.785	49.578	21.745
267	ALA	N	35.677	37.593	31.161	313	H2O	OH2	28.911	19.876	22.976
267	ALA	CA	35.566	36.560	32.179	314	H2O	OH2	29.797	51.940	35.038
267	ALA	CB	34.165	35.963	32.078	315	H2O	OH2	8.968	16.983	43.770
267	ALA	C	36.616	35.454	32.087	316	H2O	OH2	21.830	26.021	49.724
267	ALA	O	36.811	34.737	33.081	317	H2O	OH2	18.231	35.980	44.119
268	THR	N	37.257	35.279	30.927	318	H2O	OH2	17.725	35.088	15.203
268	THR	CA	38.227	34.187	30.751	319	H2O	OH2	34.481	23.007	20.146
268	THR	CB	37.888	33.276	29.515	320	H2O	OH2	19.764	37.086	46.005
268	THR	OG1	37.799	34.092	28.362	321	H2O	OH2	13.211	26.583	10.242
268	THR	CG2	36.575	32.530	29.710	322	H2O	OH2	10.729	31.502	26.207
268	THR	C	39.617	34.741	30.576	323	H2O	OH2	22.023	36.663	14.105
268	THR	O	40.534	33.996	30.218	324	H2O	OH2	26.324	19.922	21.851
269	ARG	N	39.728	36.045	30.801	325	H2O	OH2	30.661	17.697	22.182
269	ARG	CA	41.008	36.690	30.810	326	H2O	OH2	8.433	17.883	24.882
269	ARG	CB	40.656	38.156	30.839	327	H2O	OH2	32.021	21.783	19.092
269	ARG	CG	41.824	39.000	30.472	328	H2O	OH2	32.606	20.038	14.623
269	ARG	CD	41.544	40.401	29.949	329	H2O	OH2	27.918	17.370	24.830
269	ARG	NE	42.811	40.930	29.432	330	H2O	OH2	17.445	14.094	24.149
269	ARG	CZ	43.324	42.136	29.787	331	H2O	OH2	16.527	18.554	15.250
269	ARG	NH1	44.518	42.533	29.265	332	H2O	OH2	15.380	14.546	15.873
269	ARG	NH2	42.681	42.951	30.667	333	H2O	OH2	12.129	16.040	17.903
269	ARG	C	41.844	36.161	32.014	334	H2O	OH2	13.873	16.685	15.209
269	ARG	OT1	41.328	35.597	32.990	335	H2O	OH2	6.048	18.751	34.243
269	ARG	OT2	43.070	36.206	31.952	336	H2O	OH2	4.411	16.951	35.536
270	CM	CM	27.629	24.423	14.043	337	H2O	OH2	6.528	15.046	39.508
271	CM	CM	18.482	35.001	42.551	338	H2O	OH2	4.188	15.102	37.754
272	H2O	OH2	35.625	16.277	36.682	339	H2O	OH2	7.267	13.144	37.517
273	H2O	OH2	19.773	36.339	42.049	340	H2O	OH2	7.231	10.169	35.676
274	H2O	OH2	28.438	25.352	47.303	341	H2O	OH2	9.229	11.210	38.524
275	H2O	OH2	25.023	30.639	43.381	342	H2O	OH2	13.492	9.745	35.358
276	H2O	OH2	23.352	28.163	42.310	343	H2O	OH2	12.026	44.524	42.622
277	H2O	OH2	21.594	35.893	18.729	344	H2O	OH2	11.004	41.120	45.663
278	H2O	OH2	22.058	31.111	19.688	345	H2O	OH2	10.220	39.693	42.722
279	H2O	OH2	18.752	45.063	40.645	346	H2O	OH2	12.059	47.753	40.959
280	H2O	OH2	18.039	30.216	23.124	347	H2O	OH2	9.164	48.300	42.769
281	H2O	OH2	14.078	9.380	32.356	348	H2O	OH2	11.958	43.338	44.851
282	H2O	OH2	15.449	19.938	28.355	349	H2O	OH2	11.239	46.641	44.371
283	H2O	OH2	15.927	25.605	30.476	350	H2O	OH2	4.931	44.533	41.923
284	H2O	OH2	12.858	32.346	37.185	351	H2O	OH2	6.403	36.291	34.865
285	H2O	OH2	11.544	33.624	27.713	352	H2O	OH2	5.564	39.764	36.611
286	H2O	OH2	11.580	8.103	31.642	353	H2O	OH2	8.066	29.304	32.467
287	H2O	OH2	42.076	35.854	14.697	401	H2O	OH2	23.985	29.300	19.050
288	H2O	OH2	8.591	11.660	25.062	402	H2O	OH2	22.840	42.988	23.949
289	H2O	OH2	34.301	29.140	15.200	403	H2O	OH2	24.648	47.653	34.651
290	H2O	OH2	30.440	24.492	43.369	404	H2O	OH2	22.155	15.174	18.497
291	H2O	OH2	35.793	42.916	26.272	405	H2O	OH2	22.394	50.724	27.973
292	H2O	OH2	30.881	38.720	32.534	406	H2O	OH2	25.205	15.404	16.200
293	H2O	OH2	29.323	24.894	39.464	407	H2O	OH2	16.769	30.931	11.057
294	H2O	OH2	30.053	41.242	26.124	408	H2O	OH2	6.421	46.954	36.986
295	H2O	OH2	26.029	30.946	34.554	409	H2O	OH2	39.155	36.951	34.253
296	H2O	OH2	23.950	42.830	40.424	410	H2O	OH2	30.425	43.985	26.477
297	H2O	OH2	22.857	33.906	20.288	411	H2O	OH2	15.991	34.160	48.706
298	H2O	OH2	29.750	12.657	20.465	412	H2O	OH2	33.843	20.940	9.231
299	H2O	OH2	16.182	42.867	32.920	413	H2O	OH2	16.995	50.196	28.127
300	H2O	OH2	20.509	35.549	16.195	415	H2O	OH2	38.899	33.531	34.689
301	H2O	OH2	21.065	41.688	15.225	416	H2O	OH2	17.892	19.864	44.040
302	H2O	OH2	12.353	41.495	42.254	417	H2O	OH2	34.568	30.498	17.440
303	H2O	OH2	11.733	34.741	14.055	419	H2O	OH2	35.622	20.284	42.959
304	H2O	OH2	7.156	35.456	31.880	420	H2O	OH2	0.206	12.428	34.387
305	H2O	OH2	7.914	47.871	34.970	421	H2O	OH2	38.833	25.261	24.721
306	H2O	OH2	5.154	42.915	39.674	422	H2O	OH2	27.524	37.611	14.941

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FIGURE 1

423 H2O OH2	33.375	39.759	31.397	498 H2O OH2	14.394	49.504	27.686
425 H2O OH2	10.662	51.211	37.076	499 H2O OH2	39.498	21.926	32.920
426 H2O OH2	28.400	26.227	22.233	500 H2O OH2	20.574	46.516	27.909
427 H2O OH2	37.069	31.271	18.172	501 H2O OH2	41.254	36.175	22.038
428 H2O OH2	35.149	22.967	42.892	502 H2O OH2	18.615	23.589	42.251
429 H2O OH2	14.410	35.423	17.549	503 H2O OH2	23.238	48.249	18.498
430 H2O OH2	34.593	37.589	20.470	504 H2O OH2	11.027	27.025	49.749
431 H2O OH2	33.293	43.729	30.636	505 H2O OH2	6.051	28.870	41.533
432 H2O OH2	18.935	12.276	22.731	506 H2O OH2	20.329	51.097	40.041
433 H2O OH2	36.502	38.642	39.753	507 H2O OH2	34.042	46.991	33.740
434 H2O OH2	30.888	44.367	36.634	508 H2O OH2	18.800	14.484	12.899
435 H2O OH2	6.433	14.502	42.412	509 H2O OH2	23.984	14.515	28.480
436 H2O OH2	23.735	32.721	13.204	510 H2O OH2	14.955	20.395	22.995
437 H2O OH2	30.269	39.336	42.632	511 H2O OH2	31.742	13.971	22.917
438 H2O OH2	6.916	37.376	38.041	512 H2O OH2	13.014	49.698	46.176
439 H2O OH2	31.535	45.230	24.294	513 H2O OH2	3.857	17.317	43.260
440 H2O OH2	21.133	38.497	43.405	514 H2O OH2	8.348	35.692	23.895
441 H2O OH2	26.156	30.548	26.735	515 H2O OH2	9.871	28.970	29.151
442 H2O OH2	20.961	41.888	36.136	516 H2O OH2	18.301	41.737	20.959
443 H2O OH2	10.366	9.353	42.909	517 H2O OH2	10.419	21.355	11.387
444 H2O OH2	15.664	13.252	41.086	518 H2O OH2	11.150	32.989	33.268
445 H2O OH2	15.488	35.603	22.544	519 H2O OH2	43.085	38.642	27.705
446 H2O OH2	8.523	29.548	42.831	520 H2O OH2	20.416	57.764	27.758
448 H2O OH2	6.347	42.537	28.354	521 H2O OH2	40.300	29.469	52.597
449 H2O OH2	20.408	28.429	14.479				
451 H2O OH2	9.986	37.579	24.768				
452 H2O OH2	34.820	21.034	34.828				
453 H2O OH2	17.186	30.632	13.537				
454 H2O OH2	12.491	19.964	46.613				
455 H2O OH2	31.523	29.927	11.890				
456 H2O OH2	12.628	27.138	21.026				
457 H2O OH2	33.466	44.288	34.479				
458 H2O OH2	19.599	43.860	38.560				
459 H2O OH2	16.152	29.460	52.727				
460 H2O OH2	12.458	29.430	17.126				
461 H2O OH2	37.639	14.784	37.217				
462 H2O OH2	9.851	34.465	20.032				
463 H2O OH2	33.545	17.795	26.313				
464 H2O OH2	9.256	16.911	34.260				
465 H2O OH2	35.476	39.839	21.547				
467 H2O OH2	23.365	24.048	13.490				
468 H2O OH2	11.732	35.837	17.577				
469 H2O OH2	30.073	50.380	31.035				
471 H2O OH2	16.204	22.887	7.809				
472 H2O OH2	27.601	27.623	26.352				
473 H2O OH2	2.443	14.804	32.338				
474 H2O OH2	33.485	27.966	24.079				
475 H2O OH2	16.400	18.715	49.507				
476 H2O OH2	34.584	26.355	28.896				
477 H2O OH2	18.844	26.392	36.213				
478 H2O OH2	17.595	33.022	12.700				
479 H2O OH2	19.970	49.821	15.851				
480 H2O OH2	29.931	22.624	47.074				
481 H2O OH2	28.764	29.952	13.997				
482 H2O OH2	24.923	29.997	46.055				
483 H2O OH2	4.494	34.569	48.325				
484 H2O OH2	25.927	28.389	42.632				
485 H2O OH2	19.179	31.050	19.865				
486 H2O OH2	33.544	35.859	34.951				
489 H2O OH2	7.275	28.059	36.209				
490 H2O OH2	18.187	52.286	20.471				
491 H2O OH2	14.703	47.608	24.076				
492 H2O OH2	14.414	29.083	26.931				
493 H2O OH2	20.741	38.573	12.784				
494 H2O OH2	32.424	22.352	42.540				
495 H2O OH2	11.669	32.823	30.485				
496 H2O OH2	25.506	21.376	19.908				

SUBSTITUTE SHEET

FIGURE 2

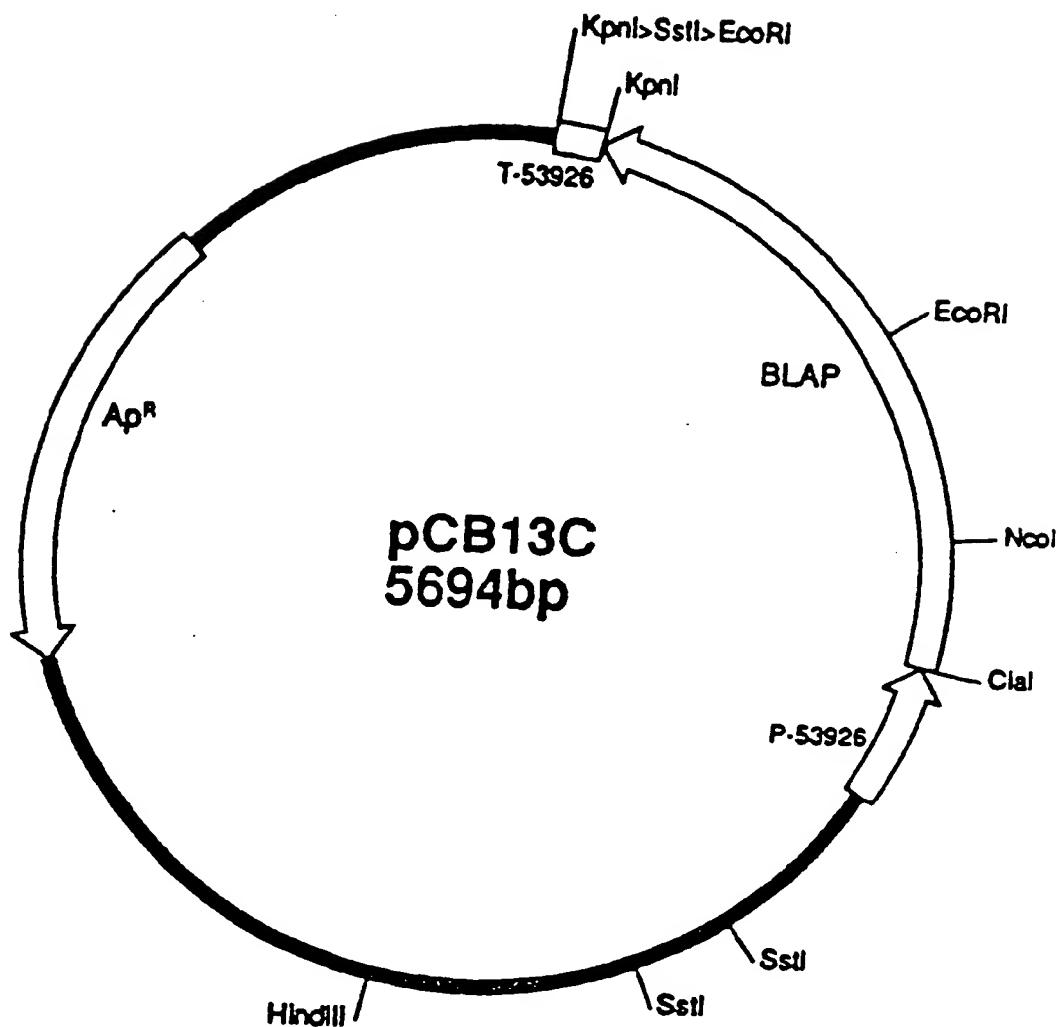
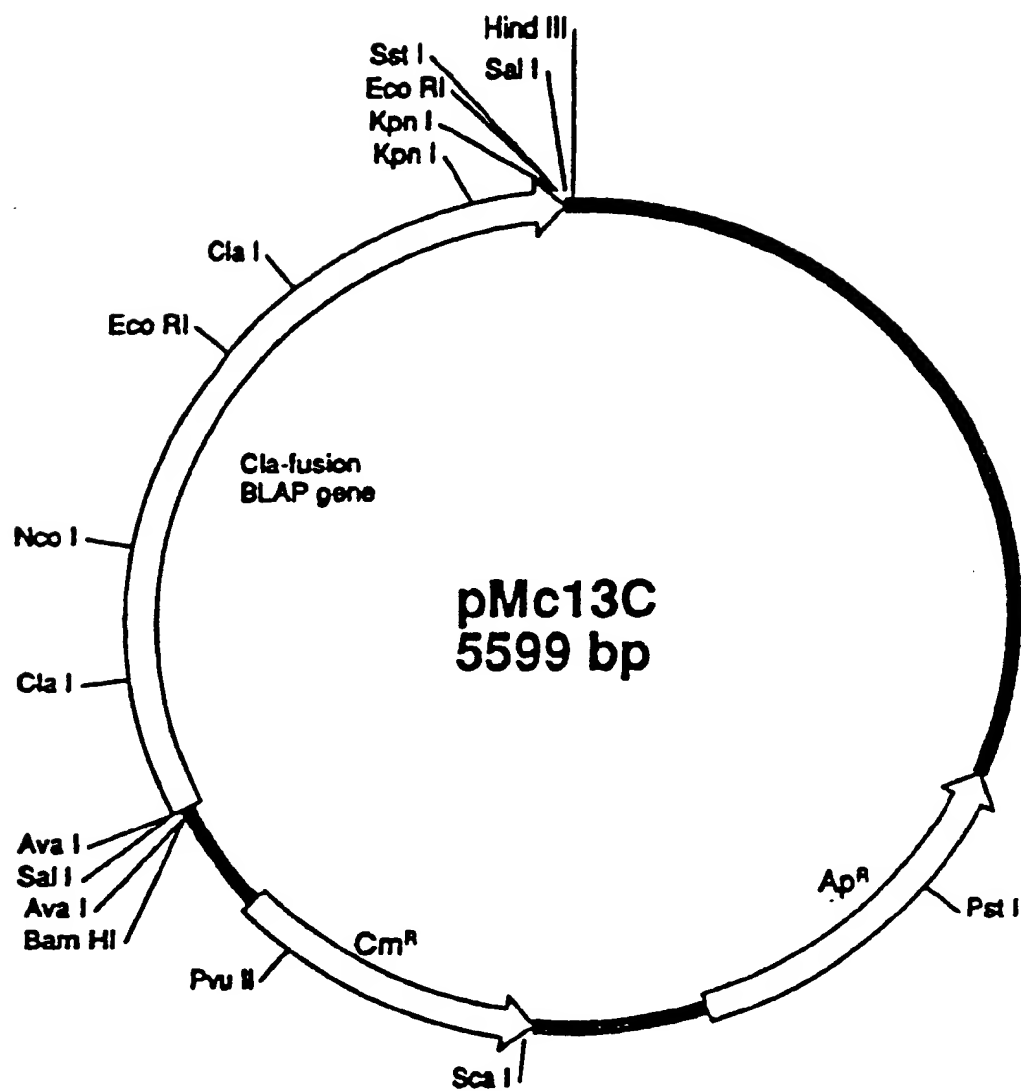


FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/04306

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/57; C12N9/54; C07K3/08; //(C12N9/54,
C12R1:07)**II. FIELDS SEARCHED**Minimum Documentation Searched⁷

Classification System

Classification Symbols

Int.Cl. 5

C12N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸**III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹**

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	WO,A,8 704 461 (AMGEN) 30 July 1987 cited in the application see abstract ---	1,29,53, 81,105, 133
A	WO,A,9 102 792 (HENKEL RESEARCH CORPORATION) 7 March 1991 see figure 1 ---	1-156
A	WO,A,8 906 279 (NOVO INDUSTRI) 13 July 1989 cited in the application see page 9 - page 17 ---	1-179
A	WO,A,8 909 830 (GENEX CORPORATION) 19 October 1989 cited in the application see page 9, paragraph 2 - page 11 ---	1-179
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¹⁰ Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 07 OCTOBER 1992	Date of Mailing of this International Search Report 26. 10. 92
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer VAN DER SCHAAL C.A.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,8 808 028 (GENEX CORPORATION) 20 October 1988 cited in the application see page 14 - page 19 ---	1-179
A	SCIENCE vol. 245, 1989, LANCASTER, PA US pages 54 - 57 W.S. SANDBERG AND T.C. TERWILLIGER 'Influence of interior packing and hydrophobicity on the stability of a protein' -----	157-197

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9204306
SA 61679**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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		US-A- 4990452	05-02-91

or more details about this annex : see Official Journal of the European Patent Office, No. 12/82